The Effects of Freezing on Marine Bacteria.

I. Quantitative Studies.

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ABSTRACT

When marine bacteria were exposed to a moderate freezing temperature, −16° C., for short periods, sea-water offered greater protection than broth media or distilled water. Reduction in number of bacteria was least severe in broth media of optimum salt-concentration and hydrogen ion concentration. During freezing in distilled water, transfer of bacteria from the crystallizing portion to the liquid part occurred. Old strains were more resistant to freezing, and previous cultivation at −3° produced more resistant strains. A higher percentage of cells were killed by freezing at lower freezing temperatures, by prolonged freezing, rapid freezing and repeated freezing. In truly super-cooled sea-water higher percentage reduction occurred than in frozen sea-water at −6.5° and −10°.

INTRODUCTION

Hilliard, Torissian and Stone (1915) enumerated the following factors as influencing the effects of freezing on bacteria: species or strain of bacteria, history and treatment previous to freezing, physical and chemical composition of the medium in which the organisms are frozen, temperature of freezing mixture, duration of freezing, abruptness of temperature change, and cultivation of the organisms subsequent to freezing.

For the various experiments reported in this paper strains of *Ps. fluorescens*, *Flav. deciduosum* and *Achromobacter*, all isolated from codfish slime, were used.

TECHNIQUE

The technique used in studies on freezing has a decided influence on the results obtained. Especially the examination of quantitative changes by means of plate counts has certain disadvantages. For example, the apparent increase of bacteria in substances such as soils and ice cream, upon freezing, has been traced to this method of examining the products. Clumps of bacteria naturally occurring in unfrozen soils, etc., on plating, are likely to form one colony only, whereas the freezing process will tend to break up these clumps, and the separated individual cells and smaller cell groups will each form colonies of their own (Prucha and Brannon 1926; Ellenberger 1919; Heinemann and Gordan 1917;

and Conn 1914). Vass (1919) found 200 per cent greater increase when frozen soil was thawed rapidly at 30° than when thawed slowly at 1°C., which he attributes to a similar breaking up of bacterial clumps.

All of our quantitative tests were made by means of plate counts, nevertheless, as the conditions in the case of emulsions of pure cultures in standard media were considered to be more favourable than in work with mixed cultures in natural habitats. Furthermore, direct microscopic counts were not feasible in a determination of the percentage survival of bacterial cells, since staining methods, such as Benians' (1918) negative congo red technique would not differentiate between living and freshly killed cells (Henrici 1923).

Uniform suspensions of the test cultures to be examined were made in suitable sterile media (8 cc. each, in uniform test tubes). Care was taken to obtain as uniform sized inocula as possible, since preliminary tests showed that the size of the inoculum has a decided influence on the amount of reduction. Upon exposing 81×10^6 cells of *Ps. fluorescens* per cc. sea-water to −16°C. for 4 minutes, a reduction of 11 per cent took place; using an inoculum of 79×10^4 cells per cc. the reduction was 30 per cent; and using only 78×10^2 cells per cc., the reduction increased to 50 per cent. Flexner (1907) observed that at 2° more *Diplococci intracellularis* survived in concentrated suspensions (in 0.85% NaCl solutions) than in weaker suspensions, after 24 hours' incubation.

Samples, each of one cc., were withdrawn from each tube after inoculation and thorough mixing, and plated in appropriate dilutions in nutrient agar. The plates were incubated at 20°C. and counted after 6 days.

The tubes were immersed in circulating brine at −16° for definite periods, and on removal were allowed to thaw at once in a large water bath at room temperature (16°-18°). Upon thorough shaking and mixing, the tubes were sampled again and plated as before.

To check upon the accuracy of the method used, including the personal factors, three parallel tubes of emulsion of *Ps. fluorescens* in sea-water were frozen at −16° for 4 minutes, and three plates made from each tube before and after freezing, with the results shown in table I.

TABLE I. Accuracy of determination of reduction of bacteria by freezing by means of plate counts (dilutions 1: 10,000)

	Number of colonies per plate		% reduction
	Before freezing	After freezing	
Tube I.....	82, 83, 73 } average: 79.3	55, 66, 44 } average: 55	30.7
Tube II.....	86, 72, 88 } average: 82	64, 63, 54 } average: 60.3	26.5
Tube III.....	74, 88, 85 } average: 82.3	68, 61, 48 } average: 59	28.3

Considering that the percentage reduction in this test was relatively low, the accuracy obtained was within the expected limits, the mean deviation amounting to 7 per cent.

EXPERIMENTAL RESULTS

Temperature of Freezing

Hilliard and Davis (1918) freezing *B. coli* in tap water at −0.5° to −6°C., concluded that the degree of cold below freezing is not a very important factor in destroying the bacteria. They observed, nevertheless, that on freezing for 3 hours in tap-water, the reduction of organisms increased gradually from 90.3 per cent at −0.5° to 99.2 per cent at −6°. Vass (1919), freezing pure cultures of *B. radicicola* at −15° and −190°, found a greater decrease in numbers at the lower temperature, the effect of length of exposure, incidentally, becoming less important with the lower range. The decrease was more gradual at −15° with increasing length of exposure, while at −190° the effect was almost immediate, *i.e.*, practically the same after 30 minutes as after 6 hours.

Berry (1933), working with frozen packs of berries, found that the reduction of the micro-organisms present (*Cladosporium*, *Penicillium*, *Oidium*, yeast and *Flavobacterium*) in 4 to 13 months, was much more severe at -2° to -10°C. (79 to 99 per cent) than at -20° (17 to 64 per cent) in airtight containers. Growth of micro-organisms was observed in non-airtight containers at -2° to -4°C. He suggested that the higher death rates at the higher temperatures may be due to the accumulation of lethal amounts of carbon dioxide from respiration of the berries permitted during the slower freezing. Similarly, Prescott, Bates and Highlands (1932) observed that in certain foods, such as strawberries and orange juice, more rapid destruction occurred at −4° to −6.6° than at the lower cold storage temperatures used, −18° to −29°.

Many investigators have studied the effect of extreme freezing temperatures upon bacteria. These, as well as the use of long periods of exposure, usually causing reduction of cells of from 90 to 99.9 per cent, are unsuitable when studying the freezing effects under different conditions. Variations due to different factors under observation are obscured when the percentage reduction of cells is uniformly high. In order to obtain smaller average reductions of the numbers of cells, from about 25 to 75 per cent, the test cultures in our experiments were generally exposed to −16° for short periods only, between 4 and 20 minutes.

In order to measure accurately the influence of the degree of cold, it should be possible to eliminate the factor of the rate of freezing altogether. It is well known that, under otherwise equal conditions, the rate of freezing increases with decreasing temperature of the freezing medium. The rate of heat transfer at each temperature would have to be controlled so as to make the rate of freezing uniform. No such means were available, unfortunately. Simultaneous exposure of the test tubes in circulating brines of different temperatures for the same length of time was the method used. While in this way all tubes were exposed to their respective freezing temperatures for equal periods, the tubes at the lowest temperatures froze first, so that the *Ps. fluorescens* cells in them were exposed in the

TABLE II. Effect of temperature of freezing on the reduction of *Ps. fluorescens* in distilled water (30 minutes exposure)

Freezing temperature °C.	Distilled water frozen in minutes	% reduction
0	not crystallized	26
− 3	25	27
− 6.5	25	35
−10	1	93
−16	1	98

crystallized medium for a longer time than the organisms in the tubes frozen at higher temperatures, as indicated in table II.

While an increasing percentage reduction of cells was obtained with lowered freezing temperature, it is quite apparent that the tubes exposed to −3° and −6.5°, which were super-cooled for the larger part of the treatment, had probably a relatively smaller reduction of cells than if no super-cooling had taken place. On the other hand, the actual rate of freezing was possibly more rapid in these tubes, as, after being super-cooled for 25 minutes, they crystallized almost immediately upon jarring.

In another series of tests after more prolonged exposure, 42 days, similar results were obtained with the *Achromobacter* strain, namely, a reduction from the initial number of 28,500,000 cells per cc. of nutrient broth to 9,792,000 cells per cc., 65.6 per cent, at −6.5°; to 40,000 cells per cc., 99.86 per cent, at −10°; and to 2070 cells per cc., 99.99 per cent, at −16°. In sea-water the number was reduced to 50,000, 99.82 per cent, at −6.5°; to 26,950, 99.90 per cent, at −10°; and to 4,290, 99.98 per cent., at −16°C.

Although the percentage of survival becomes very small, of the order of 0.01 per cent where large inocula are used, the actual numbers of survivors can be determined quite accurately. The effect of freezing temperature becomes, therefore, more significant when the actual number of surviving bacteria are considered, rather than the percentage.

SUPER-COOLING

Prudden (1887) believed that a greater destruction of *Staphylococci* takes place in super-cooled water at 15° to 25°F. (−9.4° to −3.9°C.) than in frozen water.

Maximov (1912) found that to produce death by cold in plants, ice formation is absolutely necessary as the plant cells can survive super-cooling to any degree.

In a series of experiments, the test-organisms (*Pseudomonas fluorescens*, *Flav. deciduosum* and *Achromobacter*) were exposed to −6.5° and −10°C. in sterile, filtered sea-water for prolonged periods. Some of the tubes in each case remained unfrozen and a comparison (table III) with the frozen tubes showed that with the exception of *Pseudomonas fluorescens*, after 42 days, a higher reduction in the number of cells occurred in the super-cooled sea-water than in the frozen sea-water. In the case of the *Pseudomonas fluorescens* culture, a greater destruction

of cells occurred, at both temperatures, in the frozen sea-water: 97.9 and 99.9 per cent, against 94.2 and 95.6 per cent, respectively, in the super-cooled sea-water after 42 days only. After 110 days, at −6.5°, the *Pseudomonas* culture also showed a higher reduction in the super-cooled sea-water tubes; at −10°, both were sterile.

TABLE III. Survival of bacteria in frozen and in super-cooled sea-water, at −6.5° and −10°C., respectively

Organism	Temp. (°C.)	Condition	Initial no.	Length of exposure in days		
				42	110	165
Ps. fluorescens	−6.5	frozen	70,000,000	1,450,000	48,000	6,000
		super-cooled		4,050,000	0	–
	−10	frozen		57,000	0	0
		super-cooled		3,080,000	0	0
Flav.deciduosum	−10	frozen	39,500,000	100,000	0	0
		super-cooled		40,000	0	0
Achromobacter	−6.5	frozen	28,500,000	50,000	2,100	0
		super-cooled		50,000	600	0
	−10	frozen		26,950	0	0
		super-cooled		0	–	0

These results do not support the conclusions of Hilliard and Davis (1918) that death by freezing is due to the crushing action during crystallization of the medium.

It is noteworthy that both in Berry's (1933) and Hilliard and Davis' (1918) experiments, crystallization was prevented by means of lowering the freezing point, using capillaries and sugar solutions, respectively, and not by super-cooling proper. A new factor was brought into play. In our own tests, the frozen and unfrozen culture tubes were identical, the super-cooling of the latter being accidental, or at least, unexpected. It was a case of true super-cooling.

In another paper (Hess 1934a) it has been shown that these marine bacteria show active growth on unfrozen agar at as low a temperature as −6.5°C., that is, they are in an active state of metabolism. Sherman and Cameron (1933, 1934) observed that young cells of *B. coli* in a very active state of metabolism (growth at 45°) were much more sensitive towards abrupt changes in environment (transfer to 10° causing over 95 per cent reduction in the number of organisms in 24 hours) than similar cells with a slow metabolic rate (growth at 10°). These survived transfer to 45° to the extent of 70 to 100 per cent. Our tests on the effects of super-cooling seem to confirm these results, namely, that cells in a less active state (in the frozen medium) have more vitality than similar cells in a more active state (in the super-cooled medium), at least at −6.5°. This agrees with the common observation that the death rate of bacterial cells increases with a rise in temperature, *i.e.*, with increased rate of metabolism (Hess 1934b). Keith (1913) also pointed out the importance of the rate of metabolism of the bacterial cell, as a factor determining its resistance towards freezing. The higher sensitiveness of

young cells towards inimical agencies (heat, cold, disinfectants) is assumed by Sherman and Albus (1924) and Wilson (1926) to be due to their higher rate of metabolism as compared with older cells.

Length of Exposure

As already mentioned, Vass (1919) found that freezing *B. radicicola* at −15° for increasing lengths of time resulted in a gradual decrease of cells. According to Sedgwick and Winslow (1902), a heavy reduction of *Eb. typhi* occurred during the first half-hour of freezing in water, after which the destruction proceeded fairly regularly as a function of time, 96.4 per cent dying in one week, 99.8 per cent in 3 weeks, and 100 per cent in 3 to 6 months. Park (1901) exposing typhoid bacteria to −5°, found 58 per cent reduction in one-half week; 86 per cent in one week; 92.5 per cent in 2 weeks; 99.6 per cent in 3 weeks, with a very gradual decrease of bacteria up to 22 weeks to 100 per cent reduction.

Tanner and Williamson (1928) observed that during prolonged action of a freezing temperature on yeast, the rate of death followed the curve of a monomolecular reaction, being proportional to the number of living cells.

In several tests the effect of short exposures of *Pseudomonas fluorescens* to freezing was determined.

Table IV. Effect of length of exposure at −16°C. in sea-water and distilled water on reduction of *Pseudomonas fluorescens*

Length of exposure (minutes)	% reduction of cells			
	Sea-water			Dist. water
4	32	57	36	..
8	40	71	60	71
16	60	74	41?	70
32	..	..	61	89
64	..	..	..	96
128	..	..	..	98
256	..	..	..	99.9

Reduction of bacteria by freezing at −16° in sea-water and distilled water was considerable within less than half an hour. As the changes obviously cannot be followed in the same culture, such slight irregularities as were encountered were due, no doubt, to variations in the conditions in different tubes. Probably due to the use of a lower freezing temperature, reduction was much more rapid than in the experiments of the authors referred to above.

More prolonged exposure in both sea-water and nutrient broth of the three test-organisms showed that, similarly, the number of survivors decreased with length of exposure to freezing, but that the death rate varied considerably with the different organisms, as shown in table V.

TABLE V. Effect of length of exposure to prolonged freezing at −6.5°C.

	Organism	Initial no.	Length of exposure in days				
			42	110	165	212	241
In sea-water..	*Ps. fluor.*	70,000,000	1,450,000	48,000	6,000	2,330	100
	Flav. dec.	39,500,000	100,000	700	10	0	0
	Achrom.	28,500,000	50,000	2,100	0	0	0
In nutr. broth.	*Ps. fluor.*	70,000,000	928,000	8,200	380	136	25
	Flav. dec.	39,500,000	50,000	160	0	0	0
	Achrom.	28,500,000	9,792,000	800,000	1,580,000	?	785,000

REPEATED FREEZING

A number of investigators have found that repeated freezing and thawing of bacterial cultures is very detrimental. Frankland and Frankland (1894) killed all vegetative anthrax cells by freezing them twice at −20°, while spores survived 29 alternate freezing and thawing processes unaffected. Hilliard and Davis (1918) completely killed typhoid organisms by freezing and thawing 5 times, and *B. prodigiosus* 4 times. Hilliard, Torissian and Stone (1915) observed a greater effect of intermittent freezing and thawing than of continuous freezing in water, in the case of *Es. coli*, and to a less extent with *B. subtilis*.

How far the observations of Fisher (1924), that in soils containing much colloidal matter repeated freezing and thawing tends to flocculate the colloids with a resulting reduction of the amount of bound water, may be applicable to colloidal matter in protoplasm is questionable, but suggestive.

Two experiments were carried out, freezing *Pseudomonas fluorescens* in distilled water at −16°, with 4 minutes' exposure in the first and 8 minutes' exposure in the second test. The time intervals of freezing, thawing, sampling and refreezing, etc., were kept as uniform as possible.

TABLE VI. Effect of repeated freezing (−16°C.) in distilled water on *Pseudomonas fluorescens*

Number of freezings	Number of organisms per cc. water		Total % reduction	Individual % reduction
	Before freezing	After freezing		
(4 min.)				
1st	16,500,000	9,025,000	45	45
2nd	9,025,000	5,555,000	66	38
3rd	5,555,000	3,800,000	77	32
(8 min.)				
1st	20,000,000	3,340,000	83	83
2nd	3,340,000	400,000	98	88
3rd	400,000	96,000	99.5	76
4th	96,000	16,800	99.9	82
			Average....	82.2

In the first test (4-minute exposure), where the freezing effect was relatively small, the percentage reduction decreased with increasing number of exposures. It is possible that it became constant after a certain number of freezings, but, unfortunately, as a greater reduction had been expected, the results of further freezings were lost on account of inappropriate dilutions. In the second test, at least, with more prolonged freezing, the percentage reductions during the individual freezing exposures appeared to be approximately uniform. When plotting the logarithms of the numbers of survivors against the numbers of freezings, the points came practically on a straight line, indicating that during a certain period the rate of destruction followed the logarithmic curve.

Rate of Freezing

It has already been mentioned that in freezing at different temperatures the rate of freezing increases with a drop in the freezing temperature. Practically no data are available regarding the effect of different rates of freezing at the same temperature. Almy and Field (1922), freezing fish muscle at $-10°$ in air (slowly) and in brine (rapidly), found no effect of the rate of freezing upon the number or general character of the bacterial flora on the skin and in the flesh and intestine of the fish (mackerel).

Two experiments were carried out, freezing *Pseudomonas fluorescens* at $-16°$ in sterilized sea-water by exposure to cold air and to brine.

Table VII. Effect of rate of freezing at $-16°$C. in sea-water on reduction of *Pseudomonas fluorescens*

Rate of freezing	% reduction	
	1	2
Frozen solid in 2 minutes	61	38
Frozen solid in 40 minutes	33	..
Frozen solid in 80 minutes	..	+ 7 (increase)

It is apparent that a higher percentage of bacteria were killed by rapid freezing. In the second test an apparent increase took place in the case of the slow freezing, probably due to a breaking-up of bacterial clumps.

While, as a general rule, rapid freezing causes less destruction to plant and animal cells, as only minute ice crystals are formed, the conditions in a bacterial emulsion in sea-water are sufficiently different to explain the result of greater destruction of bacterial cells with rapid freezing. On slow freezing the bacteria are gradually frozen out of the main body of ice, accumulating in the unfrozen portion (Sedgwick and Winslow 1902). On rapid freezing, on the other hand, the crystallization takes place so rapidly that the bacteria remain caught between the ice crystals, and greater destruction is considered to be due to this cause (Keith 1913). This phase is taken up subsequently.

Progress of Freezing

Sedgwick and Winslow (1902) found that on freezing the bacteria in water are not embedded among the developing ice crystals, but remain in the liquid portion of water together with any impurities,—"over 90 per cent. of the bacteria in water are not frozen into the ice at all but are physically excluded in the freezing process". Similarly, Clarke (1901) found that in the freezing of natural waters 95 per cent to 99 per cent of the bacteria are separated from the ice and remain in the water below. Keith (1913) believes that in pure water the bacteria have no protection when the whole mass becomes crystalline on freezing, so that the organisms suffer destruction, but that in foods most bacteria are extracted from the water crystals, together with other non-aqueous substances.

Pseudomonas fluorescens organisms were slowly frozen in distilled water (−16°, in air) and in successive tubes the frozen and liquid portions examined separately, by means of plate counts. While the reduction was 21 and 51 per cent in the frozen portion after 30 and 45 minutes' exposure respectively, in the liquid portion it was only 12 per cent after 30 minutes and after 45 minutes showed an increase of 1 per cent.

These results confirm the above views, that during the freezing process the bacteria are extruded from the solid into the liquid portion of the medium. While a small reduction occurred in the liquid portion in the initial stage, it was more than made up later by the transfer of cells from the crystallizing to the liquid portion.

The Hydrogen ion Concentration of the Medium

The effects of the reaction of the medium during freezing were examined by means of emulsions of *Pseudomonas fluorescens* in broth tubes, buffered at different pH. Control tubes showed that the pH of the medium had a direct effect on the bacteria during the term of the tests (total exposure of about one hour between initial sampling for plate counts before freezing and sampling after freezing). An increase took place in the more alkaline broth tubes, a decrease in the acid ones, and little change near pH 7.0. The results of three experiments are given in table VIII.

Table VIII. Effect of pH of medium in which *Pseudomonas fluorescens* organisms are frozen, as shown by the percentage reduction in their numbers.

Reaction of buffered broth (pH)	Experiment numbers			Control
	1	2	3	
6.0	98	83	21	23
6.3	97	69	16	..
6.9	89	68	11	9
7.4	97	77	4	..
8.0	93	89	26	+26

The smallest percentage reduction through freezing was uniformly observed near the neutral point. Considering that the pH of 6.0 and 6.3 had the most injurious direct effect, it becomes evident that the effect of freezing itself was most pronounced at the alkaline end of the series, where reduction was as high or higher than at the acid end, in spite of a favourable direct influence of an alkaline reaction on the organisms in the controls.

Salt Concentration

The effect of the salt concentration in broth upon the reduction of *Pseudomonas fluorescens* organisms by freezing was studied in several experiments, and the results are given in table IX.

Table IX. Effect of salt concentration of medium on reduction of *Pseudomonas fluorescens* by freezing (−16°C. 4 minutes)

% NaCl in nutrient broth	Experiment numbers			Control
	1	2	3	
0.0	92	55	85	6
0.8	..	51	74	..
1.6	..	50	74	..
3.2	52	67	30?	18
4.8	..	64	82	..
6.4	86	75	94	..
9.6	..	..	96	..
12.8 (not frozen)	96	..	99	28

The effect of freezing in the total absence of NaCl was quite as injurious as freezing in fairly high salt concentrations, so that one may conclude that salt (NaCl) acts as a protective substance in certain concentrations in the case of marine bacteria, during freezing in broth.

In a second series, the organisms were frozen in nutrient broth containing 0, 2, and 5 per cent NaCl, in distilled water and sterilized sea-water. Examinations were made after 10 minutes' freezing at −16°, after 24 hours, and after 7 days: controls were incubated at 20° for 24 hours. The treatment was so injurious to the bacteria even after 10 minutes' exposure that relatively little variation is noticeable in the different media. It is interesting to observe, however, that in this test the highest percentage reduction occurred in the broth media although they were most favourable for bacterial growth, as indicated by examination of the control tubes.

Equal amounts of the various media were inoculated with the same quantity containing 64,000,000 cells of an emulsion of the test-organism. The figures in table X represent the number of organisms in the total amount of medium in each culture.

While in distilled water a decrease of cells occurred in the control tubes, as

against large increases in the broth tubes, and even in sea-water, reduction by freezing in distilled water was less than in any of the broth tubes.

While the data shown in table V confirm better survival of bacteria in frozen sea-water than in frozen nutrient broth (2 per cent NaCl) even after prolonged exposure up to 241 days in the case of *Flav. deciduosum* and especially *Pseudomonas fluorescens*, the *Achromobacter* strain behaved directly opposite. Survival in broth was much greater, only 97.2 per cent reduction after 241 days, against 99.99 per cent reduction in 110 days and 100 per cent reduction in 165 days in sea-water.

TABLE X. Effect of freezing in distilled water, sea-water and nutrient broth of different concentrations of NaCl on reduction of *Pseudomonas fluorescens*

Medium	Initial no.	No. after exposure to −16°C. for			No. in control after 24 hours at 20°C.
		10 min.	24 hours	7 days	
Dist. water	64,000,000	2,252,000	2,211,600	.....	29,484,000
Sea-water	64,000,000	37,917,500	9,286,500	29,200	216,050,000
0% NaCl broth	64,000,000	1,491,000	254,800	22,270	551,000,000
2% " "	64,000,000	543,750	6,160	140	672,100,000
5% " "	64,000,000	202,500	13,460	108	660,000,000

Further tests with sea-water, distilled water and an equal mixture of both were made, and gave uniformly the highest percentage reduction in distilled water, average reduction of 82 per cent; the lowest reduction in sea-water, average 43 per cent; and medium reduction in the mixture, average reduction, 59 per cent.

Considering the difference in the pH of the distilled water (6.8) and sea-water (8.0), the protection towards freezing afforded the bacteria in sea-water is all the more remarkable because, according to results shown in table VIII, the destruction of bacteria in alkaline broth (pH 8.0) was greater than in neutral broth (pH 6.9).

Solutions of NaCl, C.P. and solar salt (Turks Island) in broth and in distilled water equal to the salt concentration of sea-water (3.2 per cent NaCl) and to half this strength, were used together with sterile sea-water and with nutrient broth made of sea-water, to compare the effect of the different salt types.

TABLE XI. Effect of different types of salt on reduction of *Pseudomonas fluorescens* by freezing

Amount of salt	% reduction	
	In nutrient broth	In distilled water
1.6% NaCl C.P.	....	45.7
1.6% solar salt	....	49
Sea-water (half-strength 1:1)	....	59
3.2% NaCl C.P.	47.6	43.7
3.2% solar salt	44.2	60
Sea-water (full strength 1:0)	45	43.2

The results (table XI) showed no specific differences in the effects of the different types of salt.

EFFECT OF PREVIOUS TREATMENT OF TEST ORGANISMS

Age of Cultures

Pseudomonas fluorescens organisms from agar slant cultures of different ages were used to determine the effect of the age of the culture on the percentage reduction of organisms, when frozen in sea-water at −16° in 4 minutes.

A single test seems to indicate that old cultures are less sensitive towards freezing than young ones, in accordance with increased resistance of old cultures to unfavourable conditions in general (*e.g.*, disinfectants). Using a 1-day-old culture the reduction in number of organisms was 72 per cent, while with an 8½-weeks-old culture, reduction was 41 per cent, and with a 14-weeks-old culture reduction was 0 per cent.

Previous Temperature of Incubation

Cultures of *Pseudomonas fluorescens* that had been cultivated at different temperatures for over 7 months were used to determine whether they differed in their resistance to freezing.

No significant difference was shown by organisms cultivated at 20° and 5°, 14 per cent and 16 per cent reduction respectively, but the culture from −3° incubation temperature seemed to be decidedly more resistant to freezing, showing only 5 per cent reduction. In the case of the 0° culture, an increase of 22 per cent took place, probably due to the breaking up of clumps of bacteria.

Previous Cultivation in Glucose

Cultures of the three test-organisms (*Pseudomonas fluorescens*, *Flav. deciduosum* and *Achromobacter x*) which had been subcultured for 2 months in nutrient broth containing one per cent glucose and also in nutrient broth without glucose, were frozen in sea-water for 20 minutes at −16°.

Table XII. Effect of previous cultivation in 1% glucose broth

Cultivation medium	% reduction of organisms in sea-water by freezing		
	Ps. fluorescens	*Flav. deciduosum*	*Achromobacter x*
Broth without glucose.....	15	37	26
Broth with 1% glucose.....	16	55	7

The *Achromobacter* strain, which ferments glucose strongly with the production of acid (Hess 1934a), was found to become less sensitive towards freezing after cultivation in one per cent glucose broth. The *Pseudomonas* strain, which ferments glucose only moderately, was unaffected by the cultivation in glucose, while the *Flavobacterium*, the only one not attacking glucose, was much less resistant to freezing than a strain cultivated in plain broth. Larson and

Larson (1922) found that only such bacteria as are unable to ferment glucose (*B. megatherium*) convert it to fat and fat-like substances in the cell. If the same should apply to our test-organisms, the presence of such substances must be injurious to the cold resistance of the cells.

SUMMARY

Quantitative studies, by means of plate counts, on the effects of freezing upon the percentage reduction in number of cells of marine bacteria have shown that increase in percentage reduction was caused by lower freezing temperatures, by increasing lengths of exposure, by repeated exposure to freezing, and by rapid freezing. In super-cooled sea-water a higher percentage reduction was observed, as a rule, than in frozen sea-water at the same temperature.

It has been shown that when bacteria are frozen in distilled water, transfer of organisms takes place from the crystallizing portion to the unfrozen part of the medium.

Percentage reduction by freezing was least severe in broth media that were most favourable for bacterial growth in regard to their reaction. Freezing in filtered, sterile sea-water generally offered organisms a certain degree of protection. Percentage reduction in the number of organisms was usually smaller in sea-water than in distilled water or broth media, although growth conditions were less favourable in sea-water than in broth.

Old cultures appeared less sensitive to freezing than young cells and organisms cultivated previously at a low temperature of −3°C. appeared to be more resistant than organisms grown at 5° and 20°.

Ability to ferment glucose by the *Achromobacter* strain produced more resistant strains after cultivation in one per cent glucose broth, while the non-fermenting *Flav. deciduosum* became more sensitive to freezing.

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Effect of Temperature on the Cell Length and Shape of *Bacillus vulgatus*

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ABSTRACT

B. vulgatus cells, when growing at 37° and 20°C. on agar-slants showed rapid initial temporary increase in mean length, while at 5° the increase was gradual and prolonged, with formation of very elongated cells.

At 5°, where neither spore formation nor heat destruction of the cells interfered, as occurred at 20° and 37° respectively, maximum opportunity for modification of cell shape was given.

Comparisons with data obtained on the growth rate of *B. vulgatus* at these temperatures indicate that increase in cell length occurred mainly during periods of low growth rate.

INTRODUCTION

Clark and Ruehl (1919) and Henrici (1923, 1928) have made extensive studies on the changes in the size of young bacterial cells. Richards (1928) made a similar study of young yeast cells, during the early hours of growth at 37°C. In the particular species studied, they observed increases in the size of young cells beginning after two hours, which reached a maximum in 4 to 6 hours in some cases, the size increasing two to six-fold over the "normal". Maximum size was reached during the phase of maximum growth. Size increase was accompanied by an increase in relative slenderness of the cells, resulting in more elongated forms, and by an increase in the variation of the size of the different cells.

Comparatively few data on the influence of temperature upon such changes are available. Stuart (1924) noticed giant cells, 1.2 microns x 20 to 30 microns, in fresh cultures of *Eb. typhi* grown in special media at 10° to 14°C. They regained "normal" morphological characteristics after several transplants to agar at 37°. Almquist (1917), on the other hand, observed formation of small oval rods in cultures of *Es. coli* at 1°, with return to "normal" in two hours at 34°. Henneberg (1904), measuring yeast cells grown at various temperatures, from 35° to 10°, also found them to decrease in volume with decreasing incubation temperature, the shape becoming more elongated. The latter observations were confirmed by Zikes (1920).

During a study of the influence of low temperatures on the reproduction and on the cultural characteristics of marine bacteria (Hess 1934a, 1934b), the effect of temperature on the growth, as determined by an increase in the length of the cells of *B. vulgatus*, was studied.

EXPERIMENTAL

Cell Length

A strain of *B. vulgatus* isolated from halibut intestine was used. From a young culture, grown for 14 hours at 20°C. on agar, a number of agar slants were inoculated and incubated at 37°, 20°, 5°, 0°, and −3°. Frequent examinations were made during the first 24 hours, followed by daily examinations up to 11 days' incubation and every second day up to 4 weeks.

The examinations consisted of the preparation of smears by Benians' technique (1916) (2% aqueous Congo red), which showed the organisms clearly against the blue background. The lengths of ten straight, single organisms from each of four microscopic fields of each smear were measured under a binocular

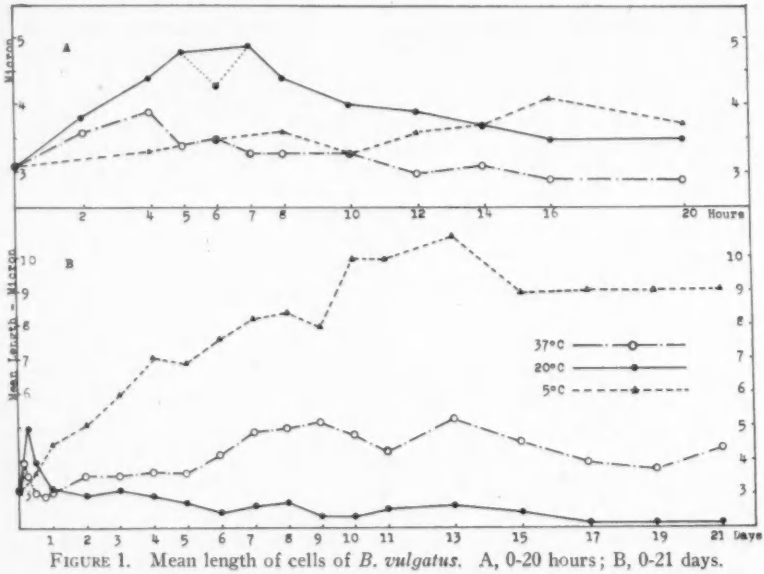

Figure 1. Mean length of cells of *B. vulgatus*. A, 0-20 hours; B, 0-21 days.

microscope by means of a standardized ocular micrometer, using a magnification of 1350 diameters. The results, each figure representing the arithmetic mean to the nearest 0.1 micron of forty measurements, are given in table I.

It is realized that the arithmetic mean of these values is possibly not the most desirable method of condensing the results of a series of 40 measurements into one figure. Robertson (1932) suggested the use of the logarithmic mean as a truer representation of the average in the case of a series of bacterial counts. Bigger and Griffiths (1933) proposed the use of a "weighted mean", which they showed to be a fairer reflection of the average observation than the logarithmic mean. In this method the result is "weighed" by paying most attention to the figures centring around the median. The difference between the arithmetic and weighted means is hardly significant, however, except where the variability of

the length of the 40 organisms becomes increasingly larger, the values at 5° after 7 days, for example, being 8.1 microns for the arithmetic mean, but only 7.4 for the weighted mean, and, after 13 days, 10.6 microns (arithmetic mean) and 9.8 (weighted mean), respectively. In the latter case, especially, the advantage of

TABLE I.—Length of cells of *B. vulgatus* at different temperatures in microns. (Arithmetic mean of 40 measurements, "weighted mean" given in brackets.)

Time of incubation (hours)	Temperature of incubation				
	37°C.	20°C.	5°C.	0°C.	−3°C.
0	3.06 (3.05)	3.06 (3.05)	3.06 (3.05)	3.06	3.06
2	3.6 (3.5)	3.8 (3.7)	—	—	—
4	3.9 (3.9)	4.4 (4.2)	3.3 (3.2)	—	3.3
5	3.4	4.8	—	—	—
6	3.5	4.3	3.5	3.6	3.5
7	3.3 (3.4)	4.9 (4.9)	—	—	—
8	3.3	4.4	3.6	3.4	3.5
10	3.3	4.0	3.3	3.2	3.1
12	3.0 (2.9)	3.9 (3.9)	3.6 (3.6)	3.7	3.5
14	3.1	3.7	3.7	3.6	3.4
16	2.9	3.5	4.1	3.2	3.0
20	2.9	3.5	3.7	3.0	3.0
24	3.0 (2.9)	3.1 (3.1)	4.4 (4.3)	3.2	3.2
36	3.2	3.2	4.5	3.5	3.1
(days)					
2	3.5 (3.4)	2.9 (2.8)	5.0 (4.9)	3.4	3.1
3	3.5	3.1	5.9	3.8	3.0
4	3.6	2.9	7.0	3.4	3.3
5	3.6	2.7	6.8	3.3	—
6	4.1	2.4	7.5	—	3.3
7	4.8 (4.8)	2.6 (2.4)	8.1 (7.4)	3.5	—
8	4.9	2.7	8.3	—	3.3
9	5.1	2.3	7.9 (7.5)	3.4	—
10	4.7 (4.3)	2.3 (2.3)	9.9 (9.8)	—	2.7
11	4.2	2.5	9.9 (9.2)	3.5	3.2
13	5.2 (5.0)	2.6 (2.5)	10.6 (9.8)	3.6	3.0
15	4.5	2.4	8.9	3.6	3.0
17	3.9	2.1	9.0	—	2.8
19	3.7 (3.6)	2.1 (2.1)	9.0 (7.8)	3.3	—
21	4.3	2.1	9.1	—	2.7
23	4.5	—	8.0 (7.3)	3.6	—
25	4.5	—	8.1 (7.4)	3.6 (3.4)	2.9 (2.8)
28	4.4 (4.1)	2.1 (2.0)	7.3 (6.6)	3.9 (3.7)	2.8 (2.6)

the weighted mean method becomes quite evident. From figure 3 it can easily be seen that the high arithmetic mean after 13 days at 5° is due to the presence of four organisms of exceptional length (from 14 to 25 microns), while the median, which corresponds approximately with the peak of the curve, lies near 8.6 microns, *i.e.*, the value of the "weighted" mean is practically only half as far from the median as that of the arithmetic mean.

Henrici (1923) used the arithmetic mean, but measured a much larger number of cells, from 250 to 400 organisms in each case, by means of projecting microphotographic slides on a screen.

The results of the present measurements are plotted in figure 1. In figure 2 the variability curves for the lengths of the 40 organisms measured in each instance are represented for the temperatures of 37° and 20°C., respectively, during the initial periods of increasing length, and shortly after reaching the maximum, when the variation in length became smallest. Figure 3 represents the variability curve for the 5° culture during the period of increasing length. A study of table I and figure 1 reveals that at 37° and 20° the findings of Clark and Ruehl

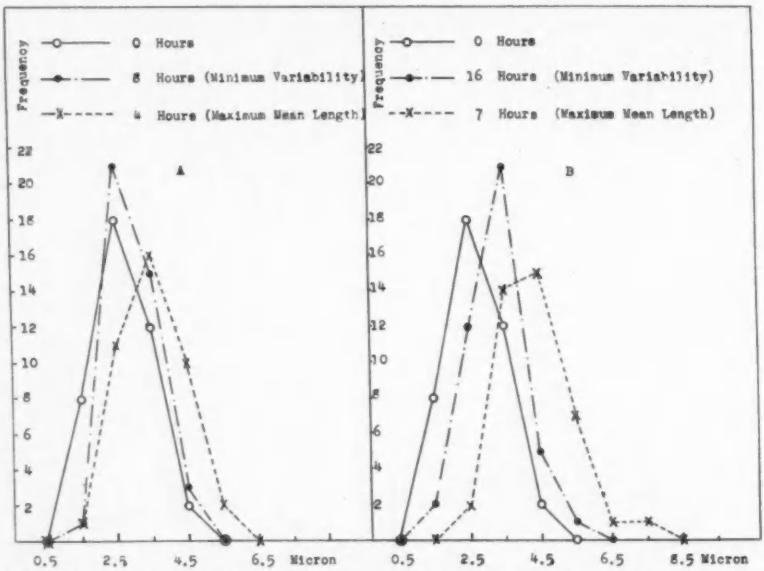

FIGURE 2. Variability of length of young cells of *B. vulgatus*. A, at 37°C.; B, at 20°C.

(1919) and Henrici (1923) were confirmed. The former found, for *B. anthracis* and *B. subtilis*, a sudden increase in size after transplantation to a new medium, with a gradual decrease to the original level after 12 hours. Henrici (1923) observed that the body volume of *Es. coli* increased tremendously during the first 3 hours, after which the volume returned to the original in 12 hours, to increase again, to a smaller extent, during the next 36 hours.

In the present tests, at 37°, the maximum mean length was reached in 4 hours, followed by a decrease to the original mean length in 12 hours. The length remained at that level up to 24 hours, after which time a gradual increase occurred for about 9 days. At 20°, a similar increase in mean length took place during the initial hours of growth, reaching its maximum somewhat later than at 37°, namely, after 7 hours. It returned gradually to its original level at 24 hours, remained

fairly constant up to 3 days, when it continued to decrease irregularly up to 3 weeks. The last period of diminishing length was closely associated with the period of maximum spore formation.

At 5°, increase in length was slow and gradual up to 13 days, with a somewhat stationary period between 8 and 20 hours.

The mean length, after 13 days' growth at 5°, was practically twice that at 37°, and four times that at 20°. The maximum values for the mean lengths attained at the three temperatures, increased also with a decrease in incubation temperature, namely, from 3.9 microns at 37°, after 4 hours, to 4.9 microns at 20°, after 7 hours, and to 10.6 microns at 5°, after 13 days. We have, apparently, a relation of time and temperature for maximum length production of *B. vulgatus*, where the Tammann (1895) principle is applicable.

At 0° and −3° no significant changes in mean length could be observed in 4 weeks.

Henrici (1923) noticed that the least variability in the size of the organisms occurred slightly after the maximum size had been attained. This is also true

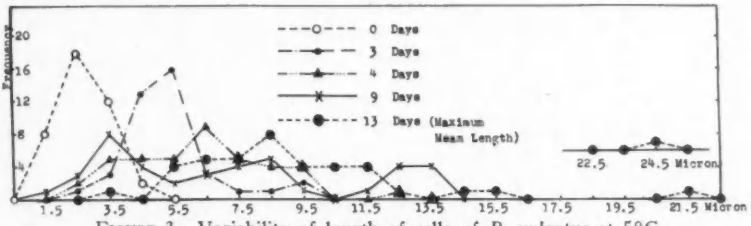

FIGURE 3. Variability of length of cells of *B. vulgatus* at 5°C.

for our results at 37° and 20°, although the delay was perhaps longer, about 4 hours at 37°, and 9 hours at 20°, between the attainment of maximum length and that of minimum variability. At 5°, the variability was lowest at the beginning, and increased steadily up to 13 days, to remain fairly constant to the end of 4 weeks. In some cases the variability curves showed more than one peak, for example, the curve at 9 days in figure 3 indicating that two different tendencies were active in the culture, one producing rods of "normal" length, peak at 3.5 microns, and another causing formation of elongated organisms, peaks at 8.5 and 12.0 microns.

Henrici (1923) found that a definite relationship existed between cell size and the rate of growth in young cultures at 37°. He made quantitative washings from uniform agar slant cultures for the preparation of smears, which were used both for the measurement of the length of the cells and for microscopic counts. He found that the maximum size was reached exactly when the maximum rate of reproduction was attained, that is, during the logarithmic growth phase. He concluded that this increase in length during initial hours of growth does not take the place of reproduction, but that the rate of body growth of the individual is greatest when the rate of reproduction is maximum and that during this period both the individual and the race are under most nearly normal conditions.

Unfortunately, in the present experiment, no simultaneous examination for changes in cell length and the rate of reproduction were carried out, so that no strictly comparable results are available.

Growth of the same strain of *B. vulgatus* was examined, however, both in buffered nutrient broth and on agar slants, at the same incubation temperatures, in separate experiments. In both cases, a 14-hour agar slant culture (20°) was used for inoculation. Comparing the results of these two tests with the previous experiment on the influence of temperature on cell length, the relation of growth rate to length of cells was found to be not as clear-cut as that observed by Henrici (1923). Obviously, this is due, partly at least, to differences in the technique employed.

In the case of cultivation in broth, the rate of reproduction was at a maximum between 6 and 7 hours at 20°. This would coincide with the time at which the maximum length of cells was reached in the previous experiments (figure 5A). At 37°, on the other hand, most rapid multiplication occurred between 4 and 6 hours.

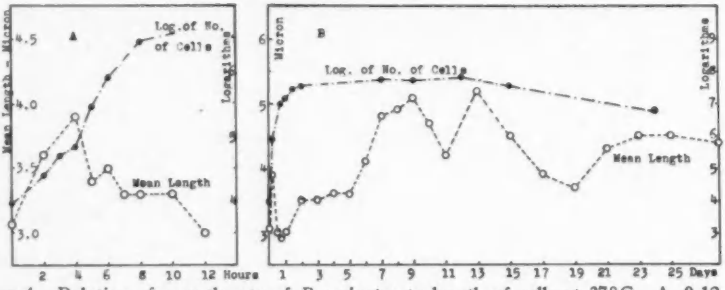

FIGURE 4. Relation of growth rate of *B. vulgatus* to length of cells at 37°C. A, 0-12 hours; B, 0-28 days.

that is, after the maximum length, had been reached in the previous experiments (figure 4A), which took four hours.

A second increase in cell length starting after 3 days, corresponds to the beginning of the phase of decrease in numbers of organisms. If the two experiments are comparable, it would indicate that at 37°, reproduction during the logarithmic phase was maintained at the cost of the size of the individual organism and that after reproduction ceased, increase in length occurred again (figure 4B).

At 5°, reproduction was practically nil up to 12 days. This coincides with the period of steadily increasing lengths of cells in the earlier experiment, so that it seems the increase in length took the place of multiplication. A sudden rapid increase in numbers occurred after 12 days, which finds its counterpart in the breaking up of the long cells obtained in the first experiment after 13 days' incubation at 5° (figure 5C).

Comparison of the rate of reproduction of agar slant cultures with the increase in the cell length on agar slants shows slightly different results. At 20° the maximum growth rate was reached only after 15 hours, so that it would seem

that here, too, the initial increase in length took the place of multiplication. Results are somewhat irregular, however, due to the errors inherent in the technique used (variations in the size of the inoculum, variations in the inoculated surface area of the slants, variations in efficiency of washing off the complete growth for plate counts). At 37°, results were similar to those in broth, the maximum rate of reproduction being from 3 to 6 hours, that is, subsequent to the time (4 hours) at which maximum cell length had been reached in the first experiment. At 5° no increase in number of cells had occurred within 12 days, from which one may again conclude that increase in cell length had taken the place of reproduction.

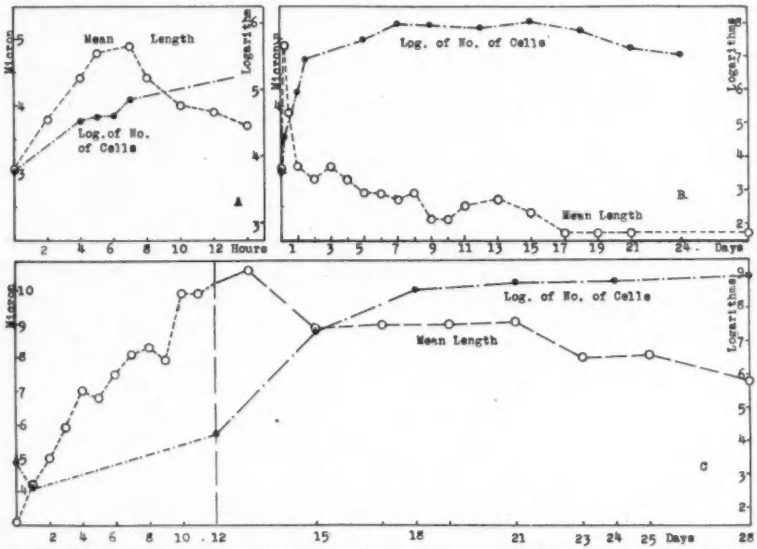

FIGURE 5. Relation of growth rate of *B. vulgatus* to length of cells. A, at 20°C., 0-14 hours; B, at 20°C., 0-28 days; C, at 5°C., 0-28 days.

The choice of *B. vulgatus* for the present experiment was perhaps unfortunate, as the factor of spore formation may have interfered to some extent, at least at 20°, where it was at a maximum.

CELL SHAPE

The agar slant cultures of *Bacillus vulgatus*, used to study the changes in length of the cells at different temperatures, furnished excellent material for investigations of the influence of temperature on the shape of the cells.

The cells were most uniform at 20°, forming straight, medium and short rods. At 37°, the cells grew to more elongated forms, many were bent and curved, and many dead cells (definitely stained, in contradistinction to the others) appeared in the smears after 3 days. This coincides with the beginning of the phase of

decrease in the broth cultures. The most interesting changes took place at 5°. The cells increased in length more than threefold, and assumed a large variety of bent and curved shapes, some assuming quite sharp angles. Simultaneously with the reaching of the maximum mean length of the cells, after 13 days, most of the larger mycelium-like threads seemed to split up into a number of medium and small, straight and slightly curved cells, this division occurring especially at the sharp angles (figure 6). Other oddly curved long threads showed, in between sections of normal width, many swollen apparently dead portions, staining with Benians' (1916) negative technique. This breaking up of the long curved threads coincides with a sudden increase in numbers of organisms in the broth cultures (figure 5C), between 12 and 18 days.

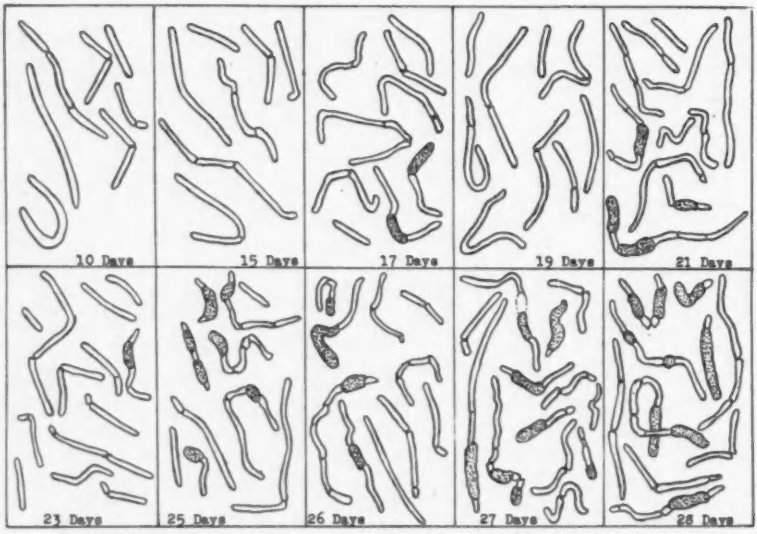

FIGURE 6. "Involution forms" of *B. vulgatus* at 5°C., (10-28 days).

We have, therefore, the least amount of modification of the shape of the cells at 20°, *i.e.* at that temperature at which the maximum spore formation takes place. This latter, apparently, interferes with any change in shape that otherwise might occur. At a higher temperature, less favourable for spore formation, the early death of the cells in its turn interferes with the formation of abnormal shapes. At a lower temperature, at 5° for example, where neither spore formation nor destruction of the cells are important factors, the maximum opportunity for modification of the shape of the cells is given.

When *B. vulgatus* was grown in nutrient broth, containing 2% NaCl, pH 7.6, small spherical heavily staining bodies were observed. They appeared more rapidly at the lower temperatures (after 43 days at −3° and −6.5°, after 63 days at 0°, and after 100 days at 20°), the ones at −3° being the largest. At this

temperature spherical bodies were again observed after 63 days and at −6.5° after 100 days. At 5° the rods were longer than at the other temperatures. Chain-formation was noticed at 13 and 45 days, after which the rods became increasingly longer, bent and curved, until after 100 days they presented a mycelium-like appearance, many showing terminal swellings (gonidia?) which stained more deeply than the rest. On removal to room temperature, the thread-like rods disappeared gradually, the staining becoming less and less intense, and small and medium straight rods made their appearance again. In the −6.5° culture, after removal to room temperature, the spherical forms had disappeared after 10 days, the culture consisting of short and medium thick rods, short chains, and formations resembling "false branching".

DISCUSSION

The phenomenon of rapid initial increase in length (and volume) is due probably to a rapid water uptake in the fresh medium. Adolph (1931), although working primarily with protozoa, states that, quite generally, size as a measure represents very closely the absolute amount of water incorporated in the individual, but only very approximately the amount of any other chemical entity. Henrici (1923) likewise attributes the subsequent decrease in body volume to the original size, to dehydration, since during that period, in spite of an increase in the number of organisms, the total bulk remains constant; in other words, the increase in bulk due to assimilation of substances from the medium is offset by a corresponding dehydration of the body substances.

The experiments showed that increase of length of the cells occurred, especially during those periods where rate of reproduction was small (at 5°, up to 13 days; and at 37°, after 2 days) in contrast to Henrici's (1923) results. Adolph (1931) pointed out that inhibition of fission by agents such as chemicals, dyes, change of pH, is generally the cause of formation of large individuals and filaments (the so-called "involution forms"). He defines adult body size as the quotient of assimilation rate and fission rate, and explains its modification by temperature, by the belief that the ratio which reproduction bears to growth in volume differs at each temperature. The regulators of the two functions are not identical. Studying the regulation of body size in the protozoan *Colpoda* at 19.3°, 21.6° and 26.5°, Adolph (1929) calculated that the relative rate of multiplication had a higher temperature coefficient ($Q_{10}=2.3$) than the relative rate of active growth per individual ($Q_{10}=1.2$). He also believes, in contrast to Henrici (1923), that fission is clearly separated from body growth, and suggests that fission is not a continuous process which goes on as growth is proceeding, but that it comes only after certain requirements have been fulfilled. The observations of Kelly and Rahn (1932) seem to confirm this view. They noticed that, especially in the case of spore-formers, the division of cell walls may be delayed after the cell contents have divided, so that it may happen that one cell divides into four cells during the stage of fastest growth. Such is no doubt the case in our experiment, where, at 5°, between 13 and 15 days, the mean length of the organ-

isms fell from its maximum value of 10.6 microns to 8.9 microns, a drop of 16 per cent., while a tremendous increase of multiplication occurred at the same time. In the microscopic smears, at the same time period, the appearance of many cell walls in the long, mycelium-like cells was noticed.

It has been shown definitely that rapid initial increase of cell length, as observed by Henrici (1923) for *Es. coli* at 37°, does not occur at all temperatures in the case of *B. vulgatus*.

A comparison of growth rate and change in size of cell, although somewhat open to criticism in our tests, also gave different results from Henrici's (1923), at various temperatures. In our experiments, growth in broth at 20° alone showed maximum cell length simultaneously with maximum growth rate, while in all other cases, increase in cell length apparently took the place of reproduction. It is questionable, also, whether the technique of quantitative examinations of agar slant cultures (by microscopic counts or plate counts) is accurate enough to warrant comparison with the changes in cell size in Henrici's (1923) investigations, any better than comparison of our plate counts of growth in broth with the increases in cell length on agar slants.

SUMMARY

The mean length of cells of *B. vulgatus*, from agar slant cultures at 37°, 20°, 5°, 0° and −3°C., were measured at frequent intervals.

The mean length showed a rapid initial increase at 37° and 20° (4 to 7 hours), followed by a decrease up to 12 to 24 hours. At 37° the second increase occurred in about 9 days, while at 20° a gradual decrease was observed simultaneously with maximum spore production.

At 5° a single gradual increase in mean length to practically three times normal took place up to 13 days, followed by rapid breaking up of the elongated cells. Separate tests of influence of temperature on the growth of *B. vulgatus* in nutrient broth and on agar slants seem to indicate that increase in cell length occurred mainly during periods of low growth rate, for example, at 5° during the first 13 days of incubation, and at 37° after 3 days' incubation. Although the comparison of such separate experiments may be questioned, these conclusions are in contrast to the results of Henrici (1923) but agree with Adolph (1931), who pointed out that inhibition of fission causes formation of large individuals and filaments.

The least amount of modification of the shape of the cells occurred at 20° at which temperature maximum spore formation took place, the latter interfering, no doubt, with any potential changes in cell shape. At 37° the early death of the cells apparently interfered with the formation of abnormal shapes, and at the low temperature (5°), where neither spore formation nor destruction of cells were important factors, and the growth rate was lowest, maximum opportunity for modification of cell shape was given.

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The Replacement of Bay of Fundy Waters

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ABSTRACT

Comparative values for the replacements of bay of Fundy waters have been determined for each year of the period 1924-1930 inclusive, by a consideration of the differences between the annual ranges of temperature at various levels in the bay of Fundy. These differences in annual ranges of temperature may be correlated to some extent with the average amount of drainage water from the St. John river system and the "adverse excess" of winds.

INTRODUCTION

The bay of Fundy, opening to the gulf of Maine and the Atlantic ocean to the southwest, is noted particularly for its large tidal amplitudes. The strong currents in the region, coupled with the configuration of the bottom, and the converging nature of the shore line, are factors which make for considerable mixing of the waters. Consequently it might be expected (Hachey 1934) that considerable differential movement will follow from the mixing of stratified waters made available from the gulf of Maine and the Atlantic ocean. Such differential movements will be enhanced somewhat by tidal mixing in the various estuaries (Hachey, unpublished MS.), and modified to some extent by wind action (Palmén 1930). Through the agency of these differential movements, replacements of waters are brought about.

THE EFFICIENCY OF THE MIXING

Temperature and salinity data obtained for the height of summer in the bay of Fundy are shown plotted in section in figure 1. The locations of the stations are shown in figure 2, and pertinent data are furnished in table I. The efficiency of the mixing is well illustrated. Comparatively stratified waters (temperatures ranging from 6.31° to 12.4° C., and salinities ranging from 32.70 ‰ to 33.47 ‰) are available at station P941 (depth 150 m.) at the mouth of the bay. From this station inwards (towards station Z16), the isotherms and isohalines are gradually displaced until, at the head of the bay (stations Z34 and Z16), they are practically vertical (indicating thorough mixing).

Temperature data for the same section, obtained for the height of winter,

TABLE I. Temperatures, salinities and densities, bay of Fundy, 1929

Station	Date	Depth (m.)	Salinity (‰)	Density	Temperature (° C.)
P941	August 21	0	32.70	24.74	12.40
"	"	25	32.83	25.14	10.80
"	"	50	33.03	25.81	7.60
"	"	75	33.08	25.98	6.65
"	"	100	33.46	26.32	6.31
P960	September 10	0	32.52	24.83	11.20
"	"	25	32.83	25.40	9.26
"	"	50	32.92	25.57	8.65
"	"	75	33.13	25.89	7.55
"	"	100	33.19	25.95	7.50
P965	September 12	0	32.61	24.74	12.05
"	"	25	32.92	25.50	9.05
"	"	50	32.99	25.66	8.40
"	"	75	33.12	25.76	8.38
P971	September 16	0	32.54	24.72	11.90
"	"	25	32.66	24.94	11.20
"	"	50	32.84	25.36	9.59
"	"	75	32.84	25.36	9.59
P667	September 19	0	31.94	23.87	13.90
"	"	25	31.92	23.82?	14.10

are plotted in figure 3. Pertinent data are furnished in table II. The change from stratified conditions at the mouth of the bay (station P941) to thoroughly mixed water at the head of the bay (stations Z34 and Z16) is not as marked (as indicated by the temperatures) as that for the height of summer.

Intense stratification may develop during the summer in neighbouring waters outside of the area of typical Fundy tides. This is probably well illustrated by a comparison of the column of water at station P941, at the mouth of the bay of Fundy, with the column of water at station 10213, off northern cape Cod (Bigelow 1924, p. 985). The salinity difference between the surface

P941, August 21/29			10213, July 19/14		
Depth (m.)	Salinity	Temperature	Depth (m.)	Salinity	Temperature
0	32.70	12.40	0	31.17	16.83
			20	31.76	9.06
25	32.83	10.80	25	31.90	8.14
			40	32.34	5.38
50	33.03	7.60			
75	33.08	6.65			
100	33.46	6.31	100	32.74	3.97

and the twenty-five metre level in the bay of Fundy (station P941) is 0.13 ‰ as compared with 0.73 ‰ off northern cape Cod (station 10213 The temperature difference between the surface and the twenty-five metre level in the bay of Fundy (station P941) is 1.60° C. as compared to 8.69° C. off northern cape Cod (station 10213). For the one hundred metre column at station P941 we have a salinity difference between the surface and the one hundred metre level of 0.76 ‰ and a temperature difference of 6.09° C. as compared to a salinity difference of 1.57 ‰ and a temperature difference of 12.86° C. for the one hundred metre column at station 10213.

TABLE II. Temperatures, bay of Fundy, 1932

Station	Date	Depth (m.)	Temperature (° C.)	Station	Date	Depth (m.)	Temperature (° C.)
P15	Feb. 27	0	3.4	P959	Feb. 25	0	3.1
"	"	10	3.3	"	"	10	3.5
"	"	25	3.6	"	"	25	3.5
"	"	50	3.6	"	"	50	3.6
"	"	125	3.6	"	"	75	3.6
"	"	190	4.9	"	"	100	3.6
				"	"	125	4.2
Z16	Feb. 15	0	−0.5	"	"	150	4.4
"	"	10	0.7				
"	"	25	0.8	P965	Feb. 22	0	1.8
				"	"	10	2.3
Z34	Feb. 15	0	1.8	"	"	25	2.3
"	"	10	2.2	"	"	50	2.3
"	"	25	2.2	"	"	75	2.3
"	"	50	2.2	"	"	100	2.3
P941	Mar. 6	0	2.5	P971	Feb. 17	0	2.4
"	"	10	2.2	"	"	10	2.6
"	"	25	2.2	"	"	25	2.7
"	"	50	2.7	"	"	50	2.9
"	"	75	3.0	"	"	75	2.9
"	"	100	4.4				
"	"	125	4.8				
"	"	150	4.8				

VERTICAL TEMPERATURE GRADIENTS

ANNUAL RANGE

By making the following reasonable assumptions for bay of Fundy waters: (1) tidal mixing is the chief means of heat transference between the various water levels, and (2) the annual variation in temperature at any depth may be represented by a sine curve, it can be shown (modification of the problem of periodic flow of heat in one dimension, p. 32, Ingersoll and Zobel 1913) that if A is the annual temperature range of the surface waters, and if B is the annual

temperature range at any depth, the vertical temperature gradient varies as $\log_e A - \log_e B$. Hence the difference between A and B is indicative of the value of the vertical temperature gradient.

In Bay of Fundy

During the period 1924-1930 inclusive, monthly records of temperatures were obtained at station P5 in the bay of Fundy (figure 2). The maximum and minimum values at different depths for each year are furnished in table III. The annual temperature ranges for each of these depths is also tabulated.

Table III. Maximum and minimum temperatures, annual ranges, and differences between the ranges at various levels, for station P5 in the bay of Fundy (h_{20} refers to the difference between the annual range at the surface and the annual range at 20 m., while h_{70} refers to the difference between the annual range at 20 m. and the annual range at 90 m.)

Year	Depth (m.)	Maximum (° C.)	Minimum (° C.)	Range (° C.)	h_{20}	h_{70}
1924	0	10.88	1.22	9.66		
	20	10.15	1.53	8.62	1.04	
	90	9.91	1.74	8.17		0.45
1925	0	11.25	1.14	10.11		
	20	10.80	1.01	9.79	0.32	
	90	9.24	1.18	8.06		1.73
1926	0	11.75	0.08	11.67		
	20	10.19	−0.10	10.29	1.38	
	90	9.68	−0.71	10.39		−0.10
1927	0	11.10	1.48	9.62		
	20	10.34	1.01	9.33	0.29	
	90	9.53	1.21	8.32		1.01
1928	0	11.91	2.56	9.35		
	20	11.35	2.32	9.03	0.32	
	90	10.06	2.71	7.35		1.68
1929	0	11.80	2.00	9.80		
	20	10.95	1.91	9.04	0.76	
	90	8.81	2.05	6.76		2.28
1930	0	12.80	0.55	12.25		
	20	11.43	0.97	10.46	1.79	
	90	11.19	1.08	10.11		0.35

The differences (h_{20}) between the annual range at the surface and the annual range at 20 m., and the differences (h_{70}) between the annual range at 20 m. and the annual range at 90 m. are plotted in figure 4(a) for each year of the period 1924-1930 inclusive. It will be noted from the plotted results that

when the value of h_{20} is small, the value of h_{70} is large, and vice versa. As these differences in range (h_{20} and h_{70}) are indicative of the vertical temperature gradients existing in a given year we may summarize with respect to the vertical temperature gradients as follows:

1. In 1924, 1926, and 1930 the vertical temperature gradient in the upper twenty metres (as indicated by h_{20}) was comparatively large, and in the lower seventy metres (as indicated by h_{70}) was comparatively small.

2. In 1925, 1927, 1928, and 1929, the vertical temperature gradient in the upper twenty metres (as indicated by h_{20}) was comparatively small, and in the lower seventy metres (as indicated by h_{70}) was comparatively large.

In Relation to Fresh Water Inflow

The mean annual discharge of the St. John river at Pokiok in cu. ft. per sec. has been determined by the Department of the Interior of Canada as follows. In 1924, 20,001; 1925, 25,587; 1926, 23,146; 1927, 26,517; 1928, 26,431; 1929, 21,796; 1930, 24,579. As the total drainage area of the St. John river is 21,500 sq. miles (Canada Year Book), and the drainage area of the St. John river above Pokiok is 15,300 sq. miles (Water Resources Paper No. 63, Dept. of the Interior, Canada), the annual discharge from the St. John river system, a river system of major importance to the bay of Fundy, into the bay can be calculated by multiplying the above figures by 1.4. The calculated values have been plotted in figure 4(b). With the exception of the year 1929, it will be noted that a comparatively small mean annual discharge from the St. John river system to the bay of Fundy may be correlated with comparatively large values of h_{20} (figure 4a) and comparatively small values of h_{70}. Again a comparatively large mean annual discharge from the St. John river system to the bay of Fundy may be correlated with comparatively small values of h_{20} and comparatively large values of h_{70} (1929 excepted).

"Adverse Excess" of Winds

The number of miles of wind from the various quadrants at Eastport (data furnished by the U.S. Weather Bureau) are furnished in table IV for each year

Table IV. Total mileage of wind from quadrants at Eastport, Me.

Quadrant	1924	1925	1926	1927	1928	1929	1930
SW	30252	28650	29346	25964	27402	28058	28151
NW	32450	27687	35071	29778	23718	23970	22252
SE	16942	19039	17067	19850	16670	16930	15001
NE	17058	15984	15946	19651	15007	13946	13925
Adverse excess							
SW minus NE	13194	12666	13400	6313	12395	15112	14226

of the period 1924-1930 inclusive. It is reasonable to suppose that winds from the northeast would enhance the removal of surface waters in the bay of Fundy, and that winds from southwest quadrant would tend to hold the surface waters within the bay. As the total milage of wind from the southwest quadrant is

always greater than the total milage of wind from the northeast quadrant (as indicated in table IV), the difference between the two in favour of the southwest will be referred to as the "adverse excess". The "adverse excess" of winds for each year of the period 1924-1930 inclusive is plotted in figure 4(b).

There is some indication (1929 excepted) that large "adverse excess" of winds (1924, 1926, and 1930) is correlated with comparatively large vertical

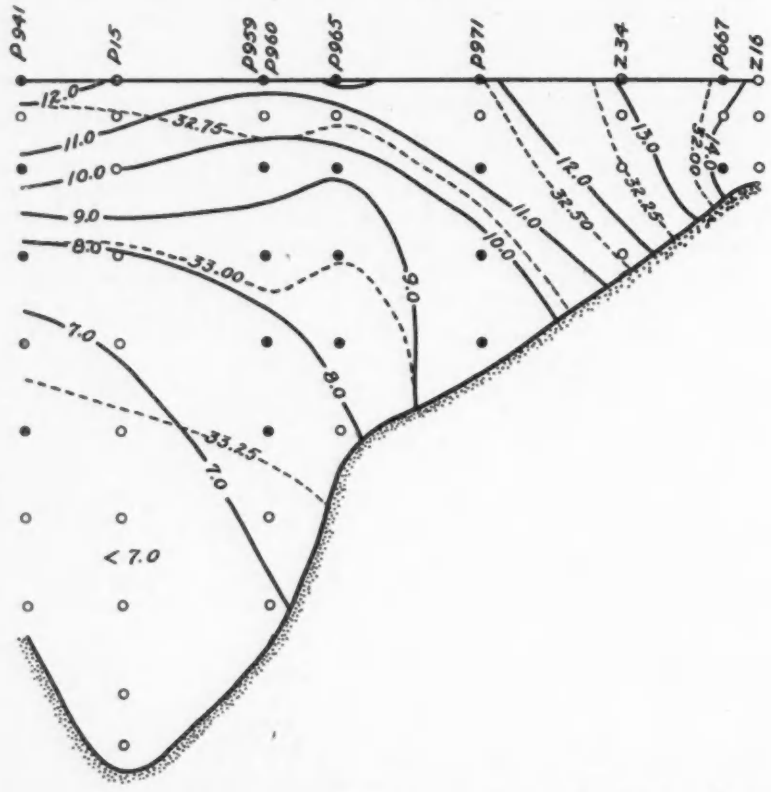

FIGURE 1. Distribution of temperature and salinity in section in the bay of Fundy. Height of summer, 1929. Observations at 0, 25, 50 metres, etc. shown thus •

temperature gradients in the upper twenty metres (h_{20}, figure 4a) and comparatively small vertical temperature gradients in the lower seventy metres (h_{70}, figure 4a), and that the smaller "adverse excess" of winds (1925, 1927, and 1928) is correlated with comparatively small vertical temperature gradients in the upper twenty metres (h_{20}, figure 4a) and comparatively large vertical temperature gradients in the lower seventy metres (h_{70}, figure 4a).

DISCUSSION

The Mechanism of Replacement

In a partially closed basin, which opens to the sea, hydrographical conditions may vary from year to year. Probably the most important factors which operate to bring about these variations in hydrographical conditions are those which control the renewal of the waters of the area.

Tidal water is carried into and out of such a basin during each successive tidal cycle. Fresh waters are carried continuously (the amount varying with the season) from the various drainage areas. Mixing of these waters produces a water which is lighter than the waters of the open sea. Dynamic gradients are

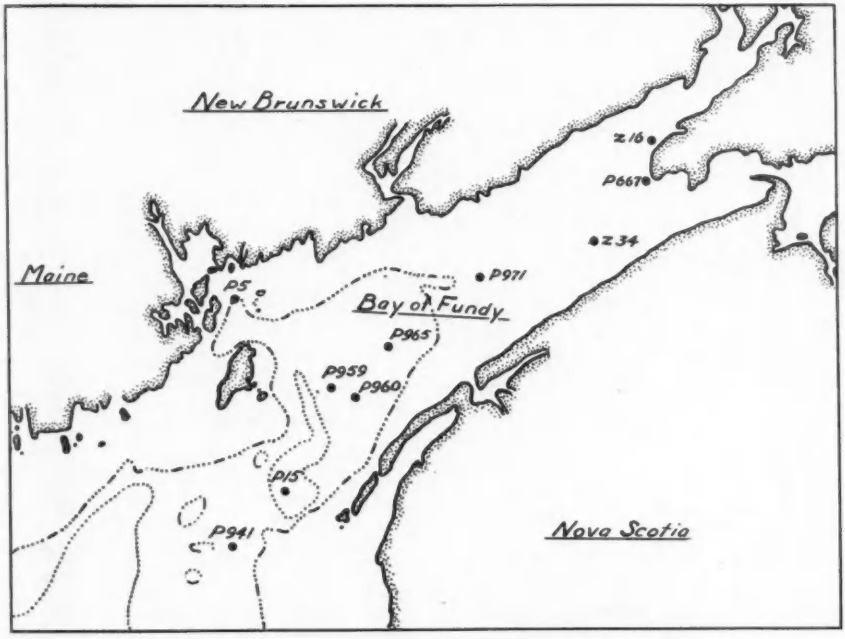

FIGURE 2. The bay of Fundy, showing locations of stations.

thus built up which tend to determine a resultant outflow from the basin in the surface layer, and a resultant inflow to the basin at the lower levels. In this manner the waters of the basin are renewed by waters from the open sea. The resultant outflow controls the rate and quantity of the resultant inflow, and consequently the renewal is controlled, in a large measure, by the factors determining the resultant outflow.

Of the many factors which tend to determine the resultant outflow from a basin (inflow of fresh water, mixing, wind action, evaporation, rainfall, heating and cooling, etc.), inflow of fresh water from river systems probably has the most

direct effect. Wind action on the other hand may also possibly prove to be of importance, for by holding the surface waters to the area, the dynamic gradients (which have been built up chiefly by the inflow of fresh water and by mixing) will be decreased in magnitude, if increased salinities should result from evaporation exceeding precipitation. On the other hand, if the winds be favourable to the outflow, the outflow of lighter surface waters will be enhanced considerably and bring about a greater resultant inflow of outside waters.

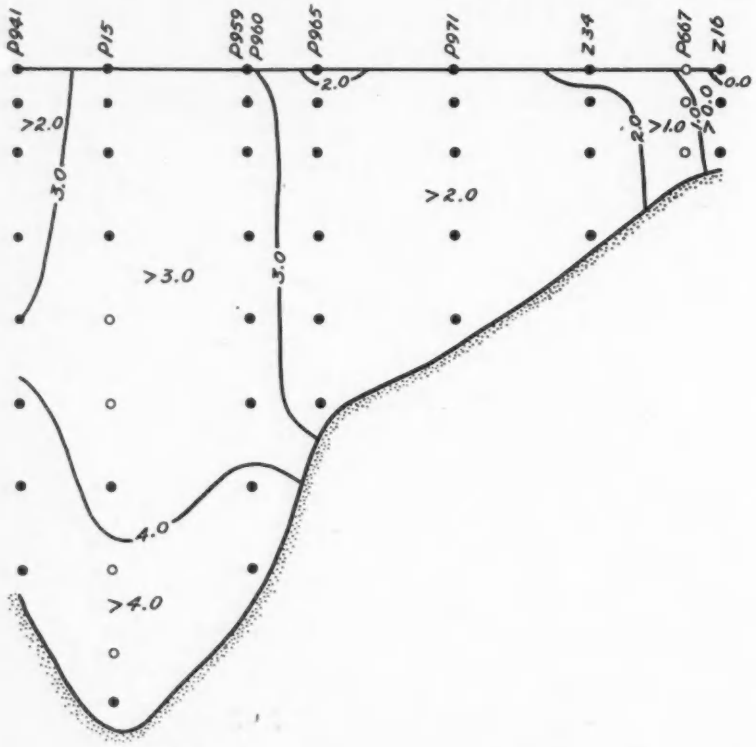

FIGURE 3. Distribution of temperature in section in the bay of Fundy. Height of winter, 1932. Observations at 0, 10, 25, 50 metres, etc. shown thus •

REPLACEMENT AS INDICATED BY VERTICAL TEMPERATURE GRADIENTS

The holding of surface waters in the bay of Fundy is favourable to the existence of a temperature gradient in the upper layers (due to the heating effect on the immediate surface in summer, and the cooling effect on the immediate surface in winter). The removal of surface waters from the bay of Fundy however, is unfavourable to the existence of a temperature gradient in the upper layers (due to resulting upwelling of comparatively cold water in summer and of

comparatively warm water in winter). The holding of surface waters to the bay of Fundy prevents renewal of the bottom waters, which in time (as a result of mixing) entails loss of a temperature gradient in the lower layers. Removal of surface waters is accompanied by renewal of the bottom waters. The bottom waters carried into the bay of Fundy are comparatively colder in summer and comparatively warmer in winter (see figures 2 and 3) than the waters of the

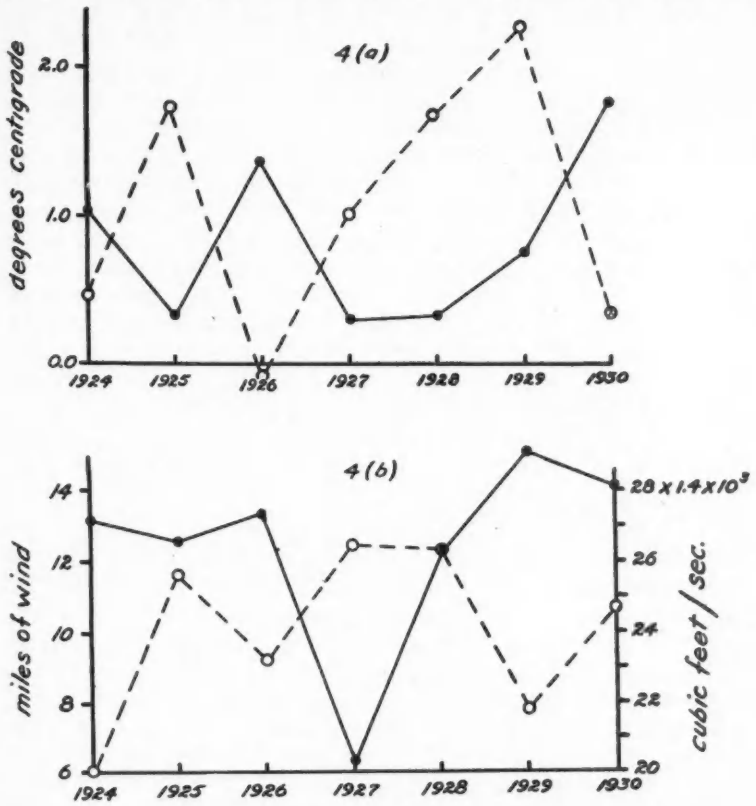

FIGURE 4. (*a*) ——— annual range of temperature at 0 m. minus the annual range of temperature at 20 m.; – – – – annual range of temperature at 20 m. minus the annual range of temperature at 90 m. (*b*) ——— "adverse excess" of winds; – – – – – average discharge from the St. John river in cu. ft./sec.

main portion of the bay. Consequently, the renewal of bottom waters is favourable to the existence of a temperature gradient in the lower levels. Hence when conditions are favourable for the existence of a temperature gradient in the upper layers (small resultant outflow of surface waters) conditions are not favourable for the existence of a temperature gradient in the lower layers (small resultant inflow of bottom waters). Again, when conditions are not favourable for the existence of a temperature gradient in the upper layers (large resultant

outflow of surface water), conditions are favourable for the existence of a temperature gradient in the lower layers (large resultant inflow of bottom waters).

Vertical Temperature Gradient in Relation to Winds

There is some indication (1929 excepted) that large "adverse excess" of winds (1924, 1926, and 1930) is correlated with comparatively large vertical temperature gradients in the upper twenty metres (h_{20}, figure 4a) and comparatively small vertical temperature gradients in the lower seventy metres (h_{70}, figure 4a) and that smaller "adverse excess" of winds (1925, 1927, and 1928) is correlated with comparatively small vertical temperature gradients in the upper twenty metres (h_{20}, figure 4a) and comparatively large vertical temperature gradients in the lower seventy metres (h_{70}, figure 4a). However, an inverse correlation seems to exist between the amount of "adverse excess" of winds and the average outflow from the St. John river, and it is thus possible that the amount of "adverse excess" is a dominant factor in determining the amount of precipitation over the drainage areas of importance to the bay of Fundy region. If such be the case, it follows that the greater the "adverse excess" of winds the smaller is the run-off from the rivers. Hence it is possible that the importance of the "adverse excess" in so far as the bay of Fundy is concerned, rests not in the resistance offered to the surface outflow, but in its control of the amount of precipitation over the various drainage areas.

Reliability of the Data

Temperature Data

As indicated in the consideration of the lengthwise section (figure 1), the bay of Fundy is an efficient mixing mechanism. Consequently, the vertical column at station 5 must reflect the hydrographic conditions of the bay of Fundy. Hence, the variations in the hydrographic conditions from year to year at station 5 may be taken as indicative of variations that have occurred in the hydrographic conditions of the bay of Fundy as a whole. It has been indicated previously that data are obtained at station 5 at monthly intervals. As the observations are usually taken about the middle of the month, the monthly temperatures should be fairly indicative of mean conditions during the month if they are to have any value. A comparison of the data at station 5 with data taken from other stations (which are occupied weekly) indicate that monthly data from station 5 are fairly representative of monthly mean conditions.

Inflow of Fresh Water

The St. John river drainage system is by far the most important source of fresh water in the bay of Fundy region. Consequently, the data would seem to be representative of the fresh water inflow to the bay of Fundy.

Wind Data

With reference to wind, the data from Eastport were used because complete data for the period dealt with was to be obtained for this station. The direction

of the wind at Eastport is not necessarily the direction of the wind over the main portion of the mouth of the bay of Fundy. Consequently, it becomes difficult to determine what portion of the data to use and what to eliminate. In opposing or enhancing the water movements, the wind sets up wind currents which veer to the right from the wind direction. Hence although data from the northeast and southwest quadrants only was made use of, it is possible that the method of treating wind data from Eastport in relation to bay of Fundy waters is not sufficiently rigorous.

CONCLUSIONS

On the basis of the preceding discussion, the vertical temperature gradients at station 5 may be made use of to derive comparative values for the replacement of bay of Fundy waters for the period 1924-1930 inclusive:

1. In the years 1924, 1926, and 1930 (see figure 4a) the difference between the annual temperature range at the surface and the annual temperature range at a depth of 20 metres was comparatively large, and the difference between the annual temperature range at a depth of 20 metres and the annual temperature range at a depth of 90 metres was comparatively small. Consequently, it would seem that replacements of bay of Fundy water took place on a comparatively small scale in the years 1924, 1926, and 1930.

2. In the years 1925, 1927, 1928, and 1929 (see figure 4a) the difference between the annual temperature range at the surface and the annual temperature range at a depth of 20 metres was comparatively small, and the difference between the annual temperature range at a depth of 20 metres and the annual temperature range at a depth of 90 metres was comparatively large. Consequently, it would seem that replacements of bay of Fundy water took place on a comparatively large scale in the years 1925, 1927, 1928, and 1929.

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Movements Resulting from Mixing of Stratified Waters

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ABSTRACT

Experiments in mixing steadily at one end of a laboratory tank an upper fresh water with a lower salt water layer demonstrate surface and bottom movements to the mixing area and an intermediate movement of mixed water away from it, which correspond with observations made in an inlet of the sea into which fresh water discharges. If fresh water is merely added at the mixing area to the tank containing salt water, the outward movement is at the surface, and there is no inward surface movement.

INTRODUCTION

In 1930 Dr. E. E. Watson studied the movements of the water at different depths in relation to Bocabec bay at the head of Passamaquoddy bay. The results showed very considerable differential movement. Dr. A. G. Huntsman suggested that a prominent feature was the movement inward or shoreward of the outer surface water and the outer deep water of very different densities, and the movement outwards at an intermediate level of the water of intermediate density resulting from the mixing of these diverse elements by the tidal currents acting over irregular, rocky bottom near shore. He suggested that the matter of the movements resulting from the mixing of stratified water could be studied to advantage in tanks in the laboratory.

In this paper certain laboratory experiments are described which illustrate the action of the dynamic gradients produced by the mixing of waters of different densities. Current observations in a mixing region illustrate the functioning of these gradients in the practical case, and lead to a consideration of the possible effect of the mixing on the circulation of the waters.

EXPERIMENTS WITH TANKS

A tank was constructed (figure 1a) consisting of a box 180 cm. long, 10 cm. wide, and 13 cm. high. At one end of the box was placed a wooden cylinder (A). 8 cm. long, 6 cm. in diameter and capable of horizontal rotation about its axis. A system of pulleys and belts connected the cylinder to an electric motor.

Experiment "A"

Fresh water was placed in the box to a depth of about 4 cm. Salt water (salinity approximately 30 $^{0}/_{00}$) was then syphoned in under the fresh water until a total depth of approximately 9 cm. was obtained. Rotation of

the cylinder A acted as a mixing mechanism at one end of the box. The water movements, following the setting up of dynamic gradients as a result of the mixing at one end of the box, were observed by means of injections of a dilution of canned milk at various levels. The action of the rotating cylinder caused considerable turbulence in its immediate vicinity. The mixed water gradually became part of a movement away from the area of mixing. Three distinct movements (figure 1b) were then noted as follows: (1) a movement in the surface layers towards the mixing area; (2) a movement in the intermediate layers away from the mixing area; and (3) a movement in the bottom layers towards the mixing area.

The amount of water moving from the mixing area will determine the amount of water drawn thereto. Hence the quantity of water carried away in the intermediate layers must be equal to the sum of the quantities carried to

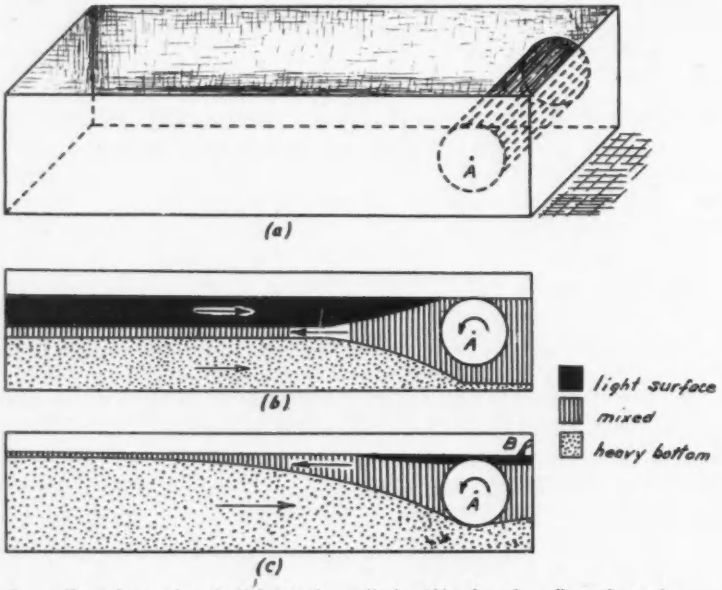

FIGURE 1. a, Experimental tank with rotating cylinder (A); b and c, effect of rotation on movements of water.

the mixing region in the surface and bottom layers. Therefore the water in the intermediate layers must either be of greater volume, or have a greater speed than that of either the surface or bottom layers taken separately. It was observed in the experiment that the water in the intermediate layer was both of greater volume and had a greater speed than the movements in either the surface or bottom layers.

The setting up of the various movements was a gradual process, due to the time required to mix the waters in the immediate vicinity of the mixing mechanism. When the maximal movements were obtained they persisted for some

time, gradually decreasing in volume and intensity, and finally dying away altogether when the waters in the box became uniform throughout, since, as the waters became less stratified, the dynamic gradients will have decreased in magnitude.

EXPERIMENT "B"

Salt water (salinity approximately 30 $^{0}/_{00}$) was placed in the tank to a depth of approximately 7 cm. A small flow of fresh water (B) was introduced in the region of the mixing mechanism (not in operation). The fresh water mixed superficially with the salt water, and the resulting water movements were observed by means of injections of diluted canned milk at various levels. A movement away from the source of fresh water took place in a thin surface layer (figure 1c), while a movement of the deeper waters towards the source of fresh water was noted. The mixing mechanism was then set in operation. The surface movement away from the mixing area was rapidly thickened and accelerated, and a corresponding thickening and acceleration was noted in the movement in the bottom layer towards the mixing area.

The amount of water drawn to the mixing area on the bottom (when the mixing mechanism is in operation) will be determined by the amount of salt water entering into the mixing and flowing away in the surface current. Hence the greater the efficiency of the mixing, the greater will be the "draw in" of the more saline waters at greater depths.

EXPERIMENT "C"

The trough was filled as in experiment "A" (light water superimposed on heavier water). The mixing mechanism was put into operation, and the time required to mix the waters was determined by noting the time at which the mixing mechanism was set in operation and the time when the system of currents died away. The time taken was approximately 14 minutes. The trough was emptied and filled again as before. A fan was used to set up a wind blowing towards the mixing mechanism. The superimposed layers became wedge shaped (figure 2a), and movements as indicated by the arrows took place (see Sandstrom 1919). The mixing mechanism also was then set in operation. The time required to mix the waters in the trough was approximately 8 minutes. It was noted that all water movements to and from the mixing mechanism (as illustrated in figure 1b) were accelerated.

The trough was emptied and filled again as before (light water superimposed on heavier water). The fan and mixing mechanism were put into operation. After a short interval, the fan was turned off. The Archimedean forces which had been built up by the wind (as the layers became wedge shaped) now set up a movement in the immediate surface layers away from the mixing area. The movement in the immediate subsurface layers towards the mixing area, the movement in the intermediate layer away from the mixing area, and the movement in the bottom layer towards the mixing area were prominent features of the system of currents set up by the operation of the mixing mechanism (figure 2b).

EXPERIMENT "D"

The trough was now filled with salt water as in experiment "B". A small flow of fresh water was introduced in the region of the mixing mechanism (not in operation). The fresh water mixed superficially with the salt waters, and movements of the waters at the different depths were as illustrated in figure 1c (surface flow away from the mixing area and a flow towards the mixing area in the bottom layers). The fan was then turned on, producing a wind blowing towards the mixing mechanism. The effect of the wind in this case was to sweep the immediate surface waters towards the mixing mechanism. Such a movement tends to cover lighter water (the fresh or slightly mixed water from the area of inflow in the region of the mixing mechanism) with heavier water (the very saline water driven by the wind). Unstable conditions result which establish vertical transference of water particles as shown (after the wind becomes

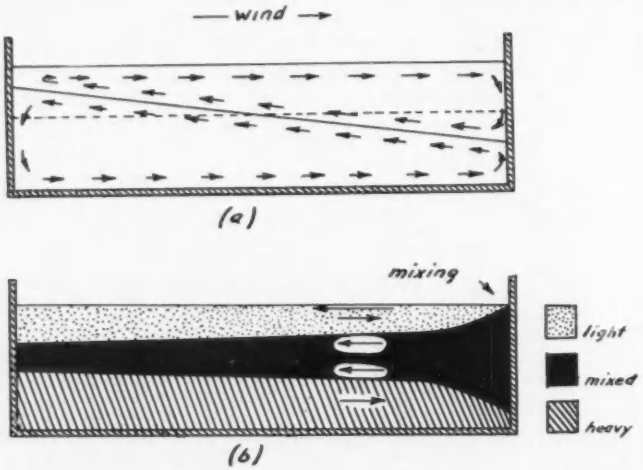

FIGURE 2. a, Effect of wind on stratified water; b, movements of water after wind has ceased to act.

steady) by the eddies set up in the immediate surface waters. The fan was then turned off and a rapid movement, away from the region of the mixing mechanism, was set up in the immediate surface layers. Movements at the greater depths were somewhat accelerated.

Repeating the above experiment (with the mixing mechanism in operation) increased the volume and intensity of the movement away from the mixing area, as well as the volume and intensity of the movement in the bottom layer towards the mixing area.

EXPERIMENT "E"

Experiments "A", "B", "C" and "D" were repeated in order to demonstrate that reversing the direction of rotation of the cylinder had no observable effect on the currents in any of the described experiments.

It has been demonstrated from these laboratory scale experiments that the mixing of stratified water sets up dynamic gradients causing the following differential movements:

(*a*) where, through the addition of fresh water at the mixing point, the mixed water is of a density which is less than that of the waters otherwise available for mixing, the mixed water is carried away from the mixing area in the upper layers, while a compensating current carries water to the mixing area in the lower layers; and

(*b*) where the mixed water is of a density which is intermediate between the densities of the surface and bottom waters available for mixing, the mixed water is carried away from the mixing area at some intermediate level, and surface and bottom waters are carried to the mixing area to compensate for the waters entering into the mixing.

A steady wind blowing towards the area of mixing is responsible for considerable modification of the above systems of currents. Such a wind seems to offer some resistance to the system outlined in (*a*), but considerably enhances a system of currents outlined in (*b*).

CURRENT OBSERVATIONS

Outstanding examples of differential movements as determined by current measurements have been furnished by Palmén (1930) for the gulf of Finland, Jacobsen (1930) for Danish waters, and de Buen (1930) and Douglas (1930) for the strait of Gibraltar. Palmén (1930) demonstrates, from observations in the gulf of Finland, an outflowing wind current in the surface strata and in the depths a compensation or slope current, setting inwards when steady easterly winds prevail. With steady. westerly winds the direction of the currents is exactly the reverse. Jacobsen (1930), from observations from various Danish lightships, and from the lightship "Schultz's Grund" at the entrance to the Great Belt, demonstrates an outgoing current in the upper sheet and an ingoing current in the lower sheet. Analogous results have been obtained at "Lappegrunden" at the entrance to the sound and in Randersfjord on the eastern coast of Jutland. In the strait of Gibraltar, it has seemingly been established that a superficial Atlantic current penetrates into the Mediterranean (de Buen 1930). The movements of the waters at the greater depths seems to be a matter of controversy. Douglas (1930) claims that the idea of an outflow (at the greater depths) from the Mediterranean to the Atlantic has long been an article of faith among oceanographers, and as a basis for his contention in favour of such a flow analyzes current measurements made in H.M.S. "Goldfinch" in 1905.

As mentioned in the introduction to this paper, Watson (1931) found considerable differential movement in the waters of Bocabec bay at the head of Passamaquoddy bay and his results probably offer the first suggestion of differential movement as a result of the mixing of stratified waters.

In Passamaquoddy bay, which opens into the bay of Fundy (figure 3), shore mixing is a matter of common personal experience. At times of river freshets, the mixed water produced in the vicinity of a river mouth in this region is of a density which is less than that of the offshore surface waters. At

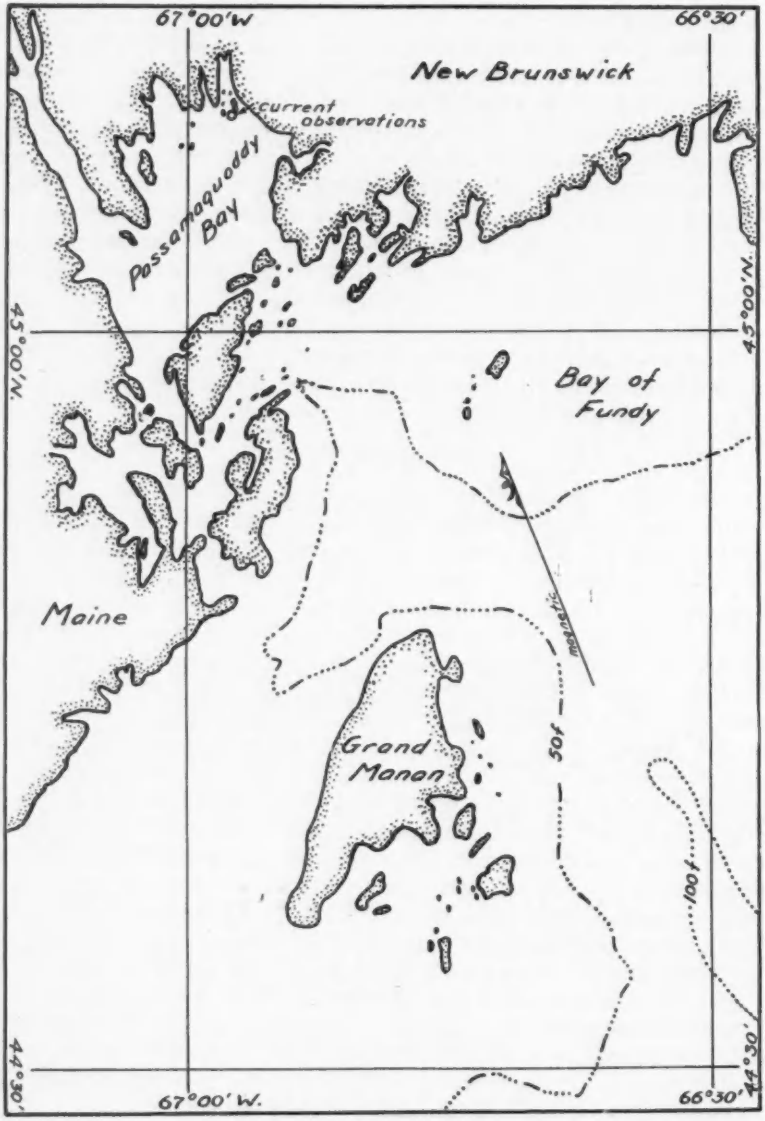

FIGURE 3. Passamaquoddy bay showing location of station for current observations.

other times when the outflow from the rivers is small, the density of the mixed water is of a value which is intermediate between that of the offshore surface waters and that of the offshore bottom waters.

Watson's observations are suggestive of a "draw in" of waters on the surface and bottom to a mixing point, and a dispersal of the mixed water at some intermediate depth. Further observations in the neighbourhood were made by the author in order to illustrate other systems of differential movements resulting from the mixing of stratified waters.

On August 18, 1933, a series of current measurements was taken throughout a complete tidal cycle at the entrance to Digdeguash harbour (figure 3). The current measurements were made with surface and depth drags similar to those used by the lightships in Finland (Witting 1930) and developed in this region by Watson (1931). The results are summarized in table I, and the north and

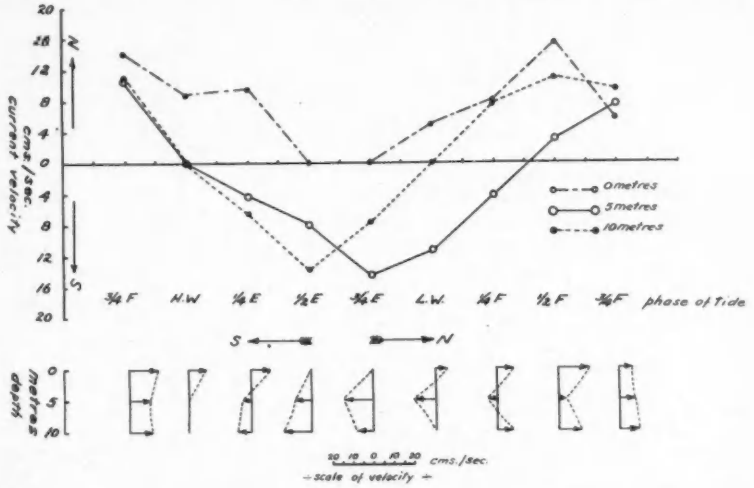

FIGURE 4. Current observations at 0, 5, and 10 metres (bottom). North and south component only. August 18, 1933.

south components are plotted in figure 4. The current as determined at a depth of 10 metres (near bottom) is practically tidal in character, turning at high water and again at low water, and reaching its maximal value at half ebb and again at half flood. The surface current was never to the south (away from the shore) due to the effect of an almost continuous fresh breeze from the south throughout the period of observation. At 5 metres, the current turned at high water, reached its maximal southerly value at three-quarters ebb, turned between one-quarter flood and half flood, and presumably reached its maximal value again at about three-quarters flood.

The average or resultant velocity (north and south components) was 7.1 cm./sec. to the north at the surface, 3.8 cm./sec. to the south at a depth of 5 metres, and practically zero at a depth of 10 metres (near bottom). The

fact that the resultant velocity at the bottom was zero, and that the currents here at this depth are seemingly tidal in character, tend to show that the only effect of the wind was to prevent the drawing of bottom water towards the shore (where the greater part of the mixing would take place). The pronounced lag

TABLE I. Current observations, August 18-1933, Digdeguash.

Time of tide	Depth (m.)	Wind	Observed current (cm./sec.)	Components of current (cm./sec.)			
				N	S	E	W
¾ flood	0	Gentle	14.3 N by W	14.0	0.0	0.0	2.8
8.27 a.m.	5	breeze	10.6 N by W	10.4	0.0	0.0	2.1
	10	S by W	10.9 N	10.9	0.0	0.0	0.0
H. W.	0	Fresh	8.6 N	8.6	0.0	0.0	0.0
9.59 a.m.	5	breeze	5.6 E	0.0	0.0	5.6	0.0
	10	S	0.0	0.0	0.0	0.0	0.0
¼ ebb	0	Fresh	9.5 N	9.5	0.0	0.0	0.0
11.31 a.m.	5	breeze	4.4 S	0.0	4.4	0.0	0.0
	10	S	6.4 S	0.0	6.4	0.0	0.0
½ ebb	0	Fresh	0.0	0.0	0.0	0.0	0.0
1.03 p.m.	5	breeze	8.3 S by E	0.0	8.1	1.6	0.0
	10	S	19.5 SE	0.0	13.8	13.8	0.0
¾ ebb	0	Fresh	0.0	0.0	0.0	0.0	0.0
2.35 p.m.	5	breeze	14.7 S by E	0.0	14.4	2.9	0.0
	10	S	7.9 S by E	0.0	7.7	1.5	0.0
L. W.	0	Fresh	5.0 N	5.0	0.0	0.0	0.0
4.07 p.m.	5	breeze	11.5 S by E	0.0	11.3	2.2	0.0
	10	S	0.0	0.0	0.0	0.0	0.0
¼ flood	0	Gentle	8.1 N	8.1	0.0	0.0	0.0
5.39 p.m.	5	breeze	6.0 SW	0.0	4.3	0.0	4.3
	10	S	7.8 N by W	7.6	0.0	0.0	1.5
½ flood	0	Moderate	15.6 N by W	15.3	0.0	0.0	3.0
7.09 p.m.	5	breeze	4.2 NW	3.0	0.0	0.0	3.0
	10	S	11.1 N by W	10.9	0.0	0.0	2.2
¾ flood	0	Gentle	6.2 NNW	5.7	0.0	0.0	2.4
8.41 p.m.	5	breeze	10.5 NW	7.5	0.0	0.0	7.5
	10	S	9.5 N by W	9.3	0.0	0.0	1.9

Resultant or average of north and south components for tidal cycle: 0 m.—7.1 cm./sec. N; 5 m.—3.8 cm./sec. S; 10 m.—0.1 cm./sec. N.

in the currents at a depth of 5 metres indicates that forces are operating which tend to produce a resultant southerly current at this depth. Consequently it may be concluded that wind action, producing a resultant shoreward current

in the immediate surface, brought about mixing in the upper levels. This mixed water was carried outwards from the shore at depths of about 5 metres.

On August 30 and 31, 1933, after a period of heavy rains which produced almost freshet conditions in the rivers, current measurements were taken in

TABLE II. Current observations, August 30-31, 1933, Digdeguash.

Time of tide	Depth (m.)	Wind	Observed current cm./sec.	Components of current cm./sec.			
				N	S	E	W
H. W.	0	Calm	13.3 S	0.0	13.3	0.0	0.0
7.25 p.m.	5		3.1 WSW	0.0	1.2	0.0	2.9
	10		3.7 WNW	1.4	0.0	0.0	3.4
¼ ebb	0	Calm	14.5 S	0.0	14.5	0.0	0.0
9.01 p.m.	5		5.6 S	0.0	5.6	0.0	0.0
	10		6.0 S by E	0.0	5.9	1.2	0.0
½ ebb	0	Calm	18.2 SSE	0.0	16.7	6.9	0.0
10.37 p.m.	5		9.0 S by E	0.0	8.8	1.8	0.0
	10		12.2 S by W	0.0	12.0	0.0	2.4
¾ ebb	0	Calm	22.7 S	0.0	22.7	0.0	0.0
12.13 p.m.	5		22.7 S	0.0	22.7	0.0	0.0
	10		18.9 S by E	0.0	18.5	3.4	0.0
L. W.	0	Calm	13.5 SSE	0.0	12.4	5.1	0.0
1.48 a.m.	5		11.8 SSE	0.0	10.9	4.5	0.0
	10		6.4 E by S	0.0	1.3	6.3	0.0
¼ flood	0	Calm	17.2 NE	12.3	0.0	12.3	0.0
3.23 a.m.	5		13.9 N	13.9	0.0	0.0	0.0
	10		14.7 N	14.7	0.0	0.0	0.0
½ flood	0	Calm	17.2 W	0.0	0.0	0.0	17.2
4.58 a.m.	5		20.0 NW	14.3	0.0	0.0	14.3
	10		16.7 NW	11.9	0.0	0.0	11.9
¾ flood	0	Calm	0.0	0.0	0.0	0.0	0.0
6.33 a.m.	5		11.2 N	11.2	0.0	0.0	0.0
	10		25.6 N	25.6	0.0	0.0	0.0
H. W.	0	Calm	8.3 NW	5.9	0.0	0.0	5.9
8.07 a.m.	5		4.1 W by S	0.0	0.8	0.0	4.0
	10		3.6 N	3.6	0.0	0.0	0.0

Resultant or average of north and south components for tidal cycle: 0 m. −7.2 cm./sec. S; 5 m. −1.2 cm./sec. S; 10 m. −2.1 cm./sec. N.

the same locality. In this case there was no wind throughout the period of observation. The results are summarized in table II, and the north and south components are plotted in figure 5. The average or resultant velocities (north and south components) are 7.2 cm./sec. to the south (away from shore) on the

surface, 1.2 cm./sec. to the south at a depth of 5 metres, and 2.1 cm./sec. to the north (towards the shore) at a depth of 10 metres. As the freshet waters from the Digdeguash river enter into the mixing (near the shore), the resulting mixture is of a density which is less than that of the surface waters off shore. Consequently, from the surface to a depth of about 5 metres, the resultant flow is outwards from the shore, while the "draw in" at the bottom is responsible for a resultant current of 2.1 cm./sec. to the north.

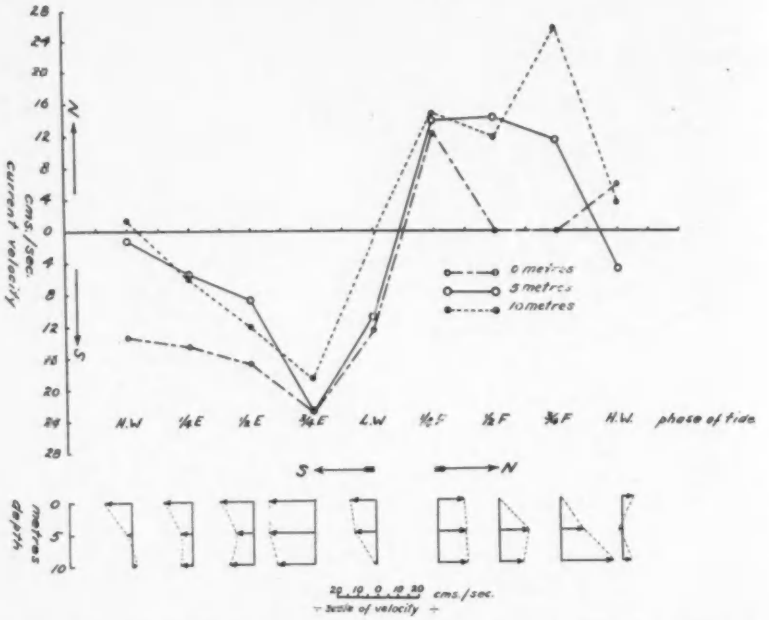

FIGURE 5. Current observations at 0, 5, and 10 metres (bottom). North and south components only. August 30-31, 1933.

DISCUSSION

It would seem that the differential movements resulting from mixing, as illustrated by laboratory experiments in tanks, have been demonstrated from the current observations in Passamaquoddy bay. Many variations in the relative movements of the layers are possible when tides, onshore winds, and mixing combine to determine them. Probably the factor of greatest magnitude in determining differential movements is the wind. This is particularly true on a windward shore. Nevertheless, it must be remembered that wind action enhances mixing, and is responsible for the storing up of large quantities of mixed water which will set up intense movements when the wind abates.

These differential movements, when of considerable magnitude, are of importance to the circulation of the waters of an area. To illustrate, let us consider two of the simpler conditions that may arise in a basin as follows.

1. Due to a large inflow of fresh waters from the rivers, the mixed water along the coast is comparatively light and tends to proceed outwards on the surface. This outflow on the surface brings about a compensating inflow at greater depths. The rotation of the earth then acts to deflect these resultant movements to the right (northern hemisphere). As a result a clockwise circulation takes place in the lower layers, and an anticlockwise rotation takes place in the upper layers.

2. Due to wind action, the surface waters are driven towards the coast, and the compensating current flows outwards at some intermediate depth. The action of the rotating earth is to set up a clockwise circulation in the upper layers, and an anticlockwise circulation in the intermediate layers.

It is a matter of common experience that considerable upwelling and mixing takes place in the region of a bank. In an area of stratified waters, such upwelling and mixing will tend to be productive of dynamic gradients which will bring about a "draw in" of surface and bottom waters, and a dispersal of mixed water at some intermediate depth. The rotating earth will tend to produce an anticlockwise circulation in the surface and bottom layers, and a clockwise circulation in the intermediate layer.

SUMMARY

1. Differential movements resulting from the mixing of stratified waters set up in an experimental tank under various conditions are comparable with observed movements under similar conditions in nature.

2. The mixing of stratified water in the sea sets up dynamic gradients causing the following systems of "making currents:" (*a*) when, through the addition of fresh water at the mixing point, the density of the mixed water is less than the density of the superficial waters outside of the mixing area, the mixed water moves away from the mixing point at the surface and a compensating current flows towards the mixing point at a deeper level; and (*b*) when the density of the mixed water at the mixing point is intermediate between the density of superficial and deeper waters outside of the mixing area, the mixed water is carried away from the mixing point at some intermediate depth, while waters are drawn to the mixing point in the surface and bottom layers.

3. Wind action on a windward shore is responsible for considerable modification of the systems of currents as outlined above (2*a* and 2*b*). Such a wind seems to offer some resistance to the system outlined in (*a*), but considerably enhances the system of currents outlined in (*b*).

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